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THE EXCRETION OF URINE IN THE DOG

VII. INORGANIC PHOSPHATE IN RELATION TO PLASMA PHOSPHATE LEVEL

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That the native, plasma "inorganic phosphate" is filterable, contrary to the opinion of Eichholtz, Robison and Brull (1925) and Eichholtz and Starling (1925), appears to be established by the observations of Walker, Ellinwood and Reisinger (1932) and White (1932) on the distribution of phosphate between the plasma and glomerular filtrate in Amphibia, and the observations of Grollman (1927) that the phosphate in mammalian plasma is completely filterable through a collodion membrane. Since the U/P ratio for phosphate may be considerably below that of other substances (in fact, urine may be almost phosphate-free at times) it is apparent that it should be considered a threshold body in Cushny's (1926) sense. Beyond these facts the available information throws little light on the mechanism of phosphate excretion. Investigators have interpreted their results in various ways, but the evidence is indirect and inconclusive (Mayrs, 1922; Underhill, 1923; White, 1923; Wigglesworth and Woodrow, 1924; Addis, Meyers and Bayer, 1925; Havard and Reay, 1926; White, 1927; Brain, Kay and Marshall, 1928).

In this paper we wish to report work on the comparison of phosphate clearances at various plasma levels with the clearances of the xylose and sucrose, substances recommended by Jolliffe, Shannon and Smith (1932) for the measurement of glomerular filtration.

A few typical experiments with xylose and phosphate are given in table 1 and the ratios of the phosphate:xylose clearance in all our experiments (77 observations) are given graphically in figure 1. We find that at normal

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plasma concentrations of phosphate (1.1 to 1.5 mM. per liter in 2 dogs) the urine is almost phosphate free,² but as the plasma phosphate is raised the phosphate clearance rises and approaches the xylose clearance; identity between the two is reached at about 9 mM. per liter, and at plasma levels of twice this value there is no tendency for the phosphate clearance to

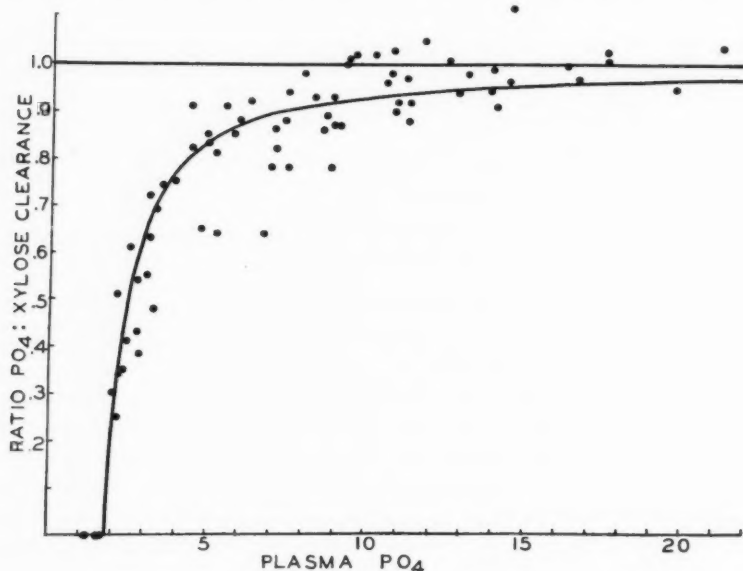


Fig. 1

exceed the xylose clearance. The curve in figure 1 is calculated by the empirical equation

$$\frac{\text{Phosphate clearance}}{\text{Xylose clearance}} = 1 - \frac{K_1}{P_{PO_4} - k_2}$$

Where P_{PO_4} is the plasma phosphate in millimols per liter, and $k_1 = 0.6$ and $k_2 = 1.2$. The constants were determined by averaging the data and we attach no significance to them at this time.

² Grollman (1927) has shown that although the normal phosphate in mammalian plasma is filterable, when the plasma calcium is elevated a considerable portion of the phosphate may be bound. Binger (1917) et al. have shown that the intravenous administration of phosphate markedly lowers the plasma calcium. It thus seems safe to assume that in these experiments in which the plasma phosphate is tremendously elevated (and by assumption the plasma calcium markedly reduced), the phosphate is in major part if not entirely filterable at the glomerulus.

TABLE 1

Comparison of phosphate and xylose clearances at varying plasma levels of phosphate

EXPERIMENT NUMBER	TOTAL CON- CURRENT TIME	URINE FLOW PER MINUTE	PHOSPHATE		XYLOSE		CM = $\frac{UV}{P} \cdot S.A.$		CM PHOSPHATE CM XYLOSE
			Plasma	Urine	Plasma	Urine	Phos- phate	Xylose	
Dog 6 (S.A. = 0.71 sq. m.)									
16	minutes	cc.	mM. per liter	mM. per liter	mgm. per 100 cc.	mgm. per 100 cc.			
	10	6.15	21.3	138.7	115	720	56.4	54.0	1.04
	20.5	6.90	17.6	117.4	124	800	64.6	62.6	1.03
	31.5	7.32	14.5	101.4	126	920	71.8	74.9	0.96
	37.0	7.35	11.2	83.0	127	965	76.4	78.6	0.97
	55.0	6.88	8.3	63.5	129	1055	73.8	79.1	0.93
Dog 4 (S.A. = 0.64 sq. m.)									
15	10	9.35	17.6	103	142	825	85.0	84.4	1.01
	22	4.58	14.0	136	128	1255	69.4	70.0	0.99
	36	4.64	11.0	116	130	1495	76.6	83.2	0.92
	49	5.73	9.0	73	127	1185	72.2	83.4	0.87
	62	6.88	7.5	50	129	1090	70.8	91.0	0.78
Dog 7 (S.A. = 0.72 sq. m.)									
26	10.5	6.34	8.95	51.4	179	1105	50.6	54.3	0.86
	21	3.43	7.45	70.2	167	1785	44.9	51.0	0.84
	36	2.23	6.31	89.6	148	2290	44.0	47.8	0.81
	49	1.96	5.55	94.6	136	2565	46.4	51.1	0.81
	60	1.91	5.00	77.9	138	2585	41.2	49.6	0.82
Dog 7									
25	11	2.63	5.24	59.7	157	2220	41.7	51.6	0.81
	22	2.73	4.46	55.9	157	2410	47.5	58.1	0.82
	35	2.69	3.93	44.1	153	2310	42.0	56.3	0.75
	47	3.25	3.55	30.7	156	1805	39.0	52.6	0.74
	64	2.41	3.36	33.2	152	2170	33.1	47.9	0.69
Dog 7									
24	11.5	4.43	3.27	11.3	207	1505	21.3	44.7	0.48
	25	3.70	2.79	9.7	198	1595	17.9	41.3	0.43
	38.5	2.67	2.44	10.4	183	1910	15.8	38.6	0.41
	42	2.04	2.19	11.3	164	2475	14.6	42.6	0.34
	53.5	1.61	2.01	9.9	151	2500	11.0	37.1	0.30

Our observations confirm Marshall (1932), who has found that in the frog the phosphate and xylose clearances are identical at high plasma phosphate levels.

Because of the theoretical significance of this result, and in view of the recent observations of White and Monaghan (1933), we have also compared the phosphate clearance with the clearance of xylose and sucrose on the one hand, and xylose and creatinine on the other, with special reference to the effect of phlorizin (Cf. tables 2 and 3). We find that in the normal dog, at high plasma phosphate levels, the phosphate clearance is identical within the limit of error indicated by the scattering of observations in figure 1 with the sugar-clearances when two sugars are present, but significantly lower than the creatinine clearance.

When phlorizin is administered, however, the phosphate clearance is reduced to levels significantly below that of xylose, glucose and creatinine.

TABLE 2
Comparison of phosphate, xylose, and sucrose clearances

EXPERIMENT NUMBER	TOTAL CONCUR- RENT TIME	URINE FLOW PER MINUTE	PHOSPHATE		XYLOSE		SUCROSE		CM = $\frac{UV}{P}$ / S. A.			CM PHOSPHATE	CM XYLOSE	CM PHOSPHATE	CM SUCROSE	CM XYLOSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Phos- phate	Xylose	Sucrose					
Dog 10 (S.A. = 0.55 sq. m.)																
82	min- utes	cc.	mM. per liter	mM. per liter	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.								
	10	2.55	14.6	94.6	144	835	133	765	30.0	26.7	26.6	1.12	1.12	1.00		
	21	1.82	12.5	87.8	139	960	145	1010	23.1	22.9	23.0	1.01	1.00	1.00		
	33	1.25	10.6	89.4	128	1130	151	1315	19.2	20.1	19.8	0.96	0.97	0.99		
												1.03	1.03	1.00		
Dog 13 (S.A. = 0.50 sq. m.)																
83	10	6.40	10.9	57.8	186	1090	122	735	67.8	75.0	77.0	0.90	0.88	1.03		
	20.5	4.76	8.9	55.6	171	1370	112	875	59.4	76.2	74.4	0.78	0.80	0.98		
	31	3.62	7.0	60.4	157	1725	105	1170	62.4	79.6	80.8	0.78	0.77	1.01		
												0.82	0.82	1.01		

White and Monaghan (1933) have concluded from observations on phlorizinized dogs that the phosphate clearance is not related to the plasma phosphate level and is absolutely inconstant. Our experiments in phlorizinized dogs perhaps explain in part White and Monaghan's observations.

Apart from the facts that phlorizin does not affect the recovery of added phosphate in plasma or urine, and that there seems to be no correlation between the dose of phlorizin and the diminution of the phosphate: xylose ratio, we can at present offer no evidence to explain the fact that this substance perturbs the phosphate clearance in manner in which it does.

In any case, the fact that the phosphate clearance approaches but never exceeds the xylose and sucrose clearance in the normal animal is, we think,

substantial confirmatory evidence that the clearance of the latter substances constitutes the true glomerular clearance. It would also appear

TABLE 3

Effect of phlorizin on the clearance of phosphate and other substances

EXPERIMENT NUMBER	TOTAL CONCUR- RENT TIME	URINE FLOW PER MINUTE	PLASMA				CM = $\frac{UV}{P} / S.A.$				CM PHOSPHATE CM Xylose	CM GLUCOSE CM Xylose	CM CREATININE CM Xylose	
			Phos- phate	Xylose	Glucose	Creati- nine	Phos- phate	Xylose	Glucose	Creati- nine				
Dog 6. Normal (S.A. = 0.71 sq. m.)														
17	min- utes	cc.	mM. per liter	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.								
	13	4.50	19.85	126		40.4	45.3	47.6		63.9	0.95		1.34	
	34	5.96	13.20	105		36.4	81.6	82.8		107.1	0.98		1.29	
	48	4.35	10.20	92.5		35.1	70.8	69.4		95.3	1.02		1.37	
										0.98			1.33	
Dog 6. Normal														
20	11	5.14	16.70	114.0		47.9	60.8	62.4		79.4	0.97		1.27	
	24	4.35	12.90	111.5		45.8	64.6	68.6		91.2	0.94		1.33	
	44	3.53	9.30	99.5		42.1	73.2	73.5		102.9	1.00		1.40	
											0.97		1.33	
Dog 6. Phlorizin (400 mgm. per kgm.)														
21	11	6.62	20.60	93.0	115.5	41.1	51.0	57.8	61.0	59.4	0.88	1.05	1.03	
	21	5.00	16.15	96.0	100.0	40.3	48.5	55.4	57.3	56.4	0.88	1.03	1.02	
	32	4.00	12.80	96.5	98.0	38.0	49.3	56.8	58.8	60.2	0.87	1.03	1.06	
											0.88	1.04	1.04	
Dog 14. Phlorizin (250 mgm. per kgm.) (S.A. = 1.5 sq. m.)														
72	10	6.95	16.0	169	118	41.2	28.5	37.8	37.6	38.1	0.74	0.99	1.01	
	21	5.82	13.0	175	112	37.7	29.7	38.1	41.1	40.3	0.78	1.08	1.06	
	31	5.10	11.2	175	100	34.7	28.4	39.1	39.9	42.6	0.73	1.02	1.09	
	42	4.14	9.8	173	103	33.0	23.3	36.2	37.3	35.6	0.67	1.03	0.98	
	61	3.42	8.5	172	117	30.8	16.0	26.6	27.6	26.9	0.60	1.04	1.01	
	81.5	3.65	7.25	168	136	29.8	13.5	24.5	27.6	24.2	0.55	1.13	0.99	
											0.68	1.05	1.02	
Dog 13. Phlorizin (50 mgm. per kgm.) (S.A. = 0.5 sq. m.)														
74	9.3	6.0	13.0	97.5	105	20.5	105	117	117	119	0.89	1.00	1.02	
	19	4.15	9.3	89.0	95	19.8	111	133	127	139	0.84	0.95	1.04	
	29.8	3.21	7.4	83.0	93	19.4	95.2	122	120	133	0.78	0.98	1.09	
											0.84	0.98	1.05	

TABLE 3—Concluded

EXPERIMENT NUMBER	TOTAL CONCUR- RENT TIME	URINE FLOW PER MINUTE	PLASMA				CM = $\frac{UV}{P} / S.A.$				CM PHOSPHATE CM XYLOSE	CM GLUCOSE CM XYLOSE	CM CREATININE CM XYLOSE	
			Phos- phate	Xylose	Glucose	Creati- nine	Phos- phate	Xylose	Glucose	Creati- nine				
Dog 9. Phlorizin (400 mgm. per kgm.) (S.A. = 0.61 sq. m.)														
75	min- utes	cc.	mM. per liter	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.								
	9 8	4.95	20.55	87.5	78.5	23.0	61.8	73.3	81.2	74.4	0.84	1.11	1.02	
	18.3	4.65	18.05	85.5	85.5	22.6	60.7	75.0	82.0	74.9	0.81	1.09	1.00	
	30.2	4.83	15.50	80.5	89.5	21.9	65.6	80.8	91.0	81.3	0.81	1.12	1.00	
											0.82	1.11	1.01	
Dog 14. Phlorizin (50 mgm. per kgm.)*														
80	10	6.25	13.4	84.0	84.5		37.4	47.2	46.2		0.79	0.98		
	22	6.25	11.0	86.5	82.0		42.8	55.0	53.8		0.78	0.98		
	34.3	5.50	9.0	87.0	76.0		42.3	53.8	55.3		0.79	1.03		
												0.79	1.00	

* In additional experiments on dog 14 which are omitted from this paper to save space, we find in alternate experiments, at corresponding plasma levels of phosphate, phosphate:xylose ratios of 0.94 and 0.91 without phlorizin and 0.82 and 0.86 with phlorizin.

from this fact that no phosphate was secreted, since the maximum phosphate clearance can be accounted for by glomerular filtration.

It is to be noted that all observations were made on a falling plasma phosphate curve, and in view of the known variability in the reabsorption of glucose (Himsworth, 1931) it is possible that the behavior of the kidney toward phosphate might be different under conditions of rising plasma phosphate.

Our observations incidentally confirm the facts, previously reported from this laboratory, that simultaneous xylose and sucrose clearances in the normal dog, and xylose, glucose and creatinine clearances in the phlorizinized dog, are identical.

METHODS. Female dogs maintained on a diet of hospital scraps were used, and all experiments were performed 18 hours after the last meal. The phosphate and sugar clearances were measured simultaneously in normal, unanesthetized dogs following the intravenous administration of a mixture of primary and secondary sodium phosphate of pH 7.4. Xylose and water were administered by stomach tube, and molar sodium phosphate in amounts from 10 to 100 cc. were infused slowly through the left external jugular vein. Ten minutes after the infusion the bladder was emptied by catheter and a blood sample drawn from the right jugular vein.

Urine collection periods were from 10 to 15 minutes in length with a blood sample at the beginning and end of each period. Blood curves were drawn and the concentrations interpolated to the exact middle of each urine period. Owing to the extremely rapid fall of the plasma phosphate concentration (approximately 50 per cent in 30 minutes) and the possibility of some urine remaining in the bladder, a deviation of ± 10 per cent in the ratios of phosphate and xylose is considered to be within the limit of error of the method.

The analyses of plasma and urine for xylose, glucose, sucrose and creatinine were carried out as described by Jolliffe, Shannon and Smith (1932) and by Shannon, Jolliffe and Smith (1932). Phosphate was determined in both blood and urine by a modification of the method of Fiske and Subbarow. Plasma phosphates were determined on a 1:5 10 per cent trichloroacetic acid filtrate. With plasma phosphate varying from 25 to 1.1 mM. per liter it is necessary to use a variable aliquot of the 1:5 filtrate, and it is also necessary to have in the final volume of 10 cc. the equivalent of 5 cc. of an 8 per cent solution of trichloroacetic acid. Consequently when an amount of filtrate less than 5 cc. was used, 8 per cent trichloroacetic acid was added to make a total of 5 cc. By this method recovery of added phosphate is possible up to 25 mM. per liter with an error of less than 2 per cent.

All phosphate analyses were performed in duplicate and in the second analysis if the phosphate was high, a quantity of plasma filtrate or urine was used so that it could be read against a 5 mM. standard. These duplicate analyses checked with an average error of considerably less than 2 per cent. As a further check on the compensation of acidity, frequent analyses were performed in triplicate; in the third analysis the blood reagent (molybdate II) was used and 5 cc. of 8 per cent trichloroacetic acid was added to the urine. These analyses also checked the preceding within ± 2 per cent.

The reagents and procedure of the original method were further modified by increasing the strength of the ammonium molybdate from 2.5 to 5.0 per cent, which increases the range over which proportionality of color is obtainable. To a total volume of unknown equalling 10 cc., 1 cc. of the molybdate reagent and 0.25 cc. of the aminonaphtholsulphonic acid were added, giving a total volume of 11.25 cc. The color was compared within 5 to 20 minutes with KH_2PO_4 standards similarly prepared and equivalent to 1 to 10 mM. per liter.

SUMMARY

In normal dogs in which inorganic phosphate has been injected intravenously the phosphate clearance is a curvilinear function of the plasma concentration. At low plasma concentrations (1.1 to 1.5 mM.) the urine

is phosphate free, but as the plasma concentration is raised the phosphate clearance rises and approaches the xylose or sucrose (glomerular) clearance without ever exceeding the latter.

This relationship does not hold in phlorizinized dogs in which the maximal phosphate clearance is depressed below the level of the simultaneous xylose, glucose and creatinine clearances, for unknown reasons.

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STUDIES ON THE CORONARY CIRCULATION

I. THE CORONARY PRESSURE PULSES AND THEIR INTERPRETATION

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A study of the coronary pressure pulses is of twofold importance: 1. The pressure in the large coronary branches constitutes the immediate pressure-head for the intramural vessels; consequently, factors which determine the absolute values or form of the pressure variations also modify the flow through the intramural vessels. 2. Deviations in the gradients or slopes of the pressure pulses in the main coronary branches might be expected if the resistance in the intramural vessels is considerably affected by contraction and relaxation of the ventricle.

Such recordings of coronary pressure have been made. W. T. Porter (1898) registered simultaneously pressure curves from the coronary and carotid artery by means of the Gad manometer. He found that their magnitudes and contours are essentially the same. This definitely disposed of the suggestion first made by Ström (1707) that the coronary orifices are occluded by the semilunar cusps during systole and seemed to warrant the deduction that the systolic, diastolic or mean pressure existing in the surface branches of the heart are practically the same as those in the aorta.

Recent studies by Hochrein and Gros (1931) raise a doubt as to the validity of such assumptions. They recorded coronary and carotid pressures with Broemser's optically recording plate-manometer. While they also found gross similarities, a careful examination of the records revealed certain differences as well, which they consider significant. Thus, 1, the coronary pressure curves have a rounded summit which contrasts with the peaked summits of the carotid pressure curves; 2, the *incisura* is barely distinguishable as a shallow dip; 3, a pronounced second peak or hump occurs after the *incisura*, etc. Furthermore, they report that systolic, diastolic and pulse pressures are generally less than in the carotid artery and do not change proportionally with those in the carotid artery when circulatory states change in a purely mechanical way. Hence, the inferences that neither the form nor actual values of the coronary pressures are solely a function of aortic pressure but are dependent also on other

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factors such as the heart rate, the aortic compression chamber, eddies and damping effects of a normally hyperplastic intima. Finally, Hochrein and Gros postulate that an equilibrium between aortic and coronary pressures cannot be quickly reached.

Though not explicitly stated it is obvious that such a conception explains observations previously made by Hochrein and his associates and independently also by Rein (1931), viz., that a consistent relation does not necessarily exist between aortic pressure and mean coronary flow and that the relative rates of systolic and diastolic flow are variable and unpredictable in the intact heart.

The validity of such deductions from the optically recorded pressure curves presented in the illustrations must be questioned however. In the first place, no exact agreement as to form and numerical values would be expected in pressure variations of the coronary and carotid arteries, for the fundamental forms and superimposed characteristics of the pressure pulse are modified considerably by the time it reaches the carotid artery (cf. Wiggers, 1928). Furthermore, in this, as in other dynamic studies, one must not rest content with the description and interpretation of optical records but must minutely evaluate the qualifications of the apparatus and the conditions of the experiment under which they were used. Frank has repeatedly warned us that highly efficient recording manometers can easily be rendered inadequate by inattention to details in their mode of application. Frank has also taught us that regardless of the sincere beliefs or claims of experimenters, technical deficiencies are unfailingly and permanently written into the records. Now a mere tyro in the field of record evaluation should be able to confirm our statement that in not a single published record does the carotid pulse display a characteristic contour. If only an equal degree of error was incurred in the registration of coronary pressure pulses, none of the conclusions reached are justified. But there is abundant reason to believe that the coronary pressure curves were seriously distorted by movements of the heart. Indeed, it is much easier to introduce such artefacts than it is to avoid them.

Absolute and convincing evidence as to whether the form and magnitude of the coronary pressure pulses passively follow those in the aorta or whether they are also affected by other factors can only be established by recording simultaneously coronary and *aortic* pressures by means of instruments of adequate efficiency under conditions of actual use and by a technique which obviates distortions due to movements of the heart. We have succeeded in recording such tracings. Their analysis which proves to be quite simple is the chief object of this report.

Procedure. Wiggers' universal optical manometers, calibrated under static conditions in relation to a base line were employed. Just before each registration their natural frequency was also determined. The

cannula of one optical manometer was inserted via the left subclavian artery so that its end lay near the semilunar valves in the aorta. The frequency of the entire system was 172 per second. The cannula of a second manometer connected to a flexible inelastic lead tube was inserted first into a lateral branch of the anterior descending ramus of the left coronary, thus recording lateral pressure in the ramus. The frequency of this system was 148. After these pressure variations had been recorded satisfactorily another cannula was inserted into the central end of the descending ramus and connected rigidly with the optical manometer, thus recording its central end pressure.² The frequency of this manometer system was 158 per sec. Since the latter procedure deprived a considerable portion of the left ventricle of its nutrient fluid and increased the possibility that its contraction is impaired, this region was perfused with a stream of oxygenated Locke's solution under low pressure through the lateral-branch cannula still remaining in position.

The registration of pressures from branches of the coronary vessel which move with the ventricle, by means of a manometer system that must necessarily have only rigid connections and remain in alignment also with the projection lamps and photokymograph presents obvious difficulties. Briefly, they were overcome in the following manner: In the first place, a branch was selected in the region midway between base and apex where the least movement seemed to occur after the pericardium had been slit. After separation of the selected branch from its sheath and placement of suitable ligatures for tying the cannulae, the largest cannula that could be inserted was selected from a collection and connected rigidly to the lead tube of the manometer. The coronary vessel was then carefully aligned with the cannula by rotating or otherwise moving the whole animal by means of a rigid and adjustable mechanical table, the details of which need not be described. Final adjustment was made by exerting vertical or horizontal traction on the pericardium by means of strings which could be securely fastened. This accomplished, the peripheral end of the branch was tied and an incision made for introducing the cannula. This was facilitated by placing a small flat supporting instrument with a handle beneath the vessel, as illustrated in figure 1. This not only steadies the vessel and allows a sufficient control of hemorrhage by traction but as it also contains a groove equal approximately to one-third that of the vessel circumference, it is possible to cut down on the plate with the assurance that an incision of proper size is made and that complete severance of the vessel is avoided. While a small stream of anticoagulant is flowing from the cannula aligned with the incised vessel, the final connection is completed,

² Owing to the length of elastic coronary vessel between the cannula and its branching from the main stem of the left coronary artery, the lateral pressure in the circumflex ramus is not actually recorded, as claimed by Porter.

not by pushing the cannula into the artery but by drawing the vessel over the end of the cannula and tying it in position. This is fairly easily accomplished through coördinated practice of two experimenters, which involved manipulation of the vessel by the small support and loose ligature and careful traction on strings attached to the pericardium.

Final adjustment is made to assure exact linear and perpendicular alignment during all phases of the cardiac cycle, but the final crucial test is given by the records only. The closest scrutiny of records published with this paper and many others in our possession bears witness to the fact that this was accomplished.

Description of records. Segments A, B and C of figure 2 give a good idea of the pressure variations recorded from a lateral branch of the anterior descending ramus of the left coronary artery. They show all the trans-

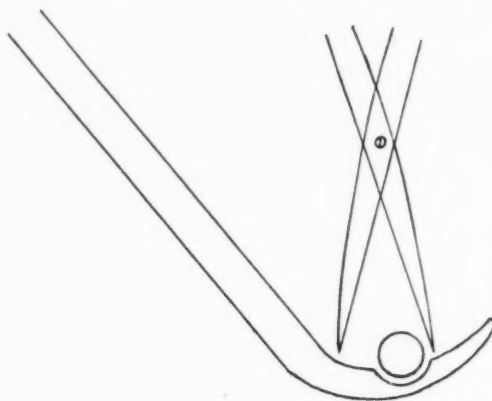


Fig. 1

formations in form that may be expected in any small branch of the aorta and with a minor variation, to be discussed later, only such. As is marked out on a long vagal beat (segment A), a slight and somewhat variable delay occurs in all the superimposed vibrations which are transmitted (*b* to *f*). A primary oscillation (*c*) is added just as in the subclavian artery (Wiggers, 1921), but if this be subtracted (dotted line), the aortic and coronary curves have identical contours during ejection. There is no indication of a more gradually rising gradient, or of additional superimposed waves such as would be expected if ventricular contraction compressed the intramural vessels significantly during the ejection phase, as maintained by Anrep and his associates (1931). The preliminary vibration (*b*), the incisura (*e*) and its after-vibration (*f*) are neither damped nor flattened, but on the contrary both are accentuated, just as is the case in the subclavian pulse

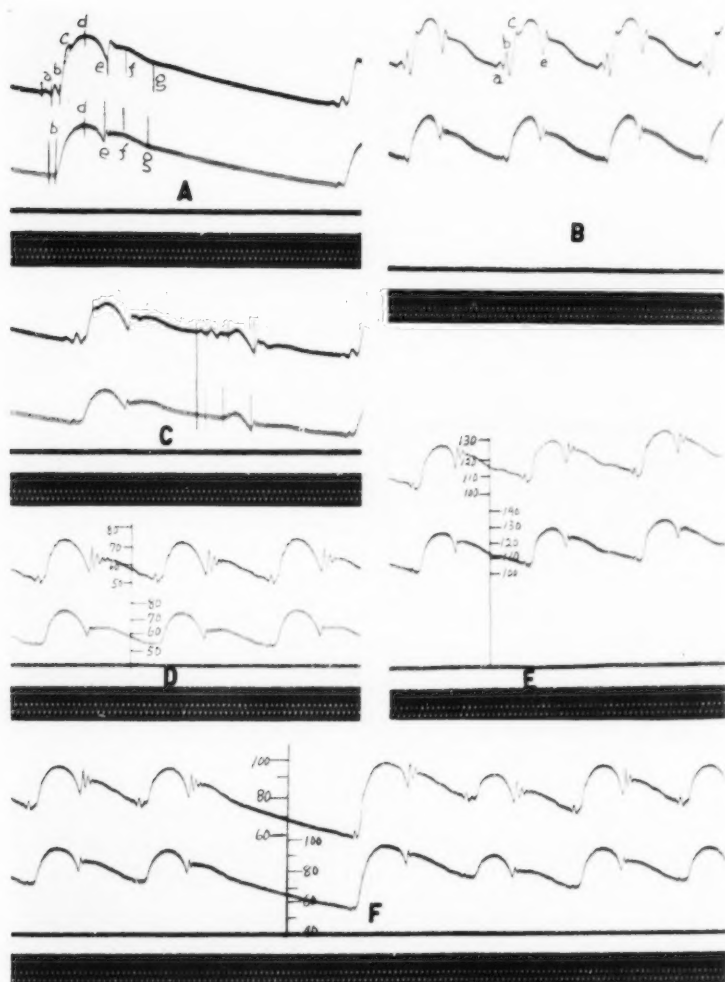


Fig. 2. Pressure pulses in coronary artery (upper) and aorta (lower record), A, B, C, coronary lateral pressures; D, E, F, coronary end pressures. Discussion in text. Time 0.02 second. (expt. C-568.)

(Wiggers, 1921). The post-incisural waves (*g* and *h*) are also clearly related to similar deflections on the descending limb of the aortic pressure pulse. In short, not a single indication exists that the form of the coronary pressure curve is affected by any of the factors postulated by Hochrein

and Gros (1931). This remains the case when circulatory conditions are altered experimentally. In segment C an almost perfect correspondence between aortic and coronary pulses is shown during a premature right ventricular contraction. If cardiac action becomes exceedingly vigorous as for instance during the rise of pressure attending temporary asphyxiation (segment B), the second preliminary oscillation (*b*), the primary oscillation (*c*) and the incisura (*d*) become amplified. The similarity of form is also retained during circulatory changes produced by central and peripheral vagus stimulation, injections of small doses of epinephrin, synephrine, amyl nitrite and hemorrhage.

The coronary lateral pressures differ essentially from those in the aorta by the occurrence of two, not one, preliminary vibration. (Cf. segments, A, B and C.) The first of these (*a*) which has no counterpart in the aortic curve begins 0.05-0.06 before the rise of aortic pressure, indicating its origin in the peripheral coronary vessels. This inference is further supported by the fact that no such oscillation occurs in pressures recorded from the central end of the descending ramus anterior (fig. 2, D, E). We are justified therefore in concluding that the significant effect of muscular contraction on intramuscular vessels is probably started before ejection begins. Records of end pressures (D, E) show an even greater similarity to aortic pressures owing to the fact that the superimposed vibrations are amplified less, indeed the primary oscillation (*c*) is only barely perceptible. These differences are explainable on simple physical grounds, but the possibility cannot be entirely excluded that an effect of muscular contraction extends over the entire isometric contraction and very slightly into the period of injection. Certainly no evidence exists that it extends any farther.

We have extended our studies also to quantitative measurements of systolic and diastolic pressure relations. Application of calibration scales made by static tests shows a close agreement, as shown in the illustrating records. Since this also is contrary to the findings of Hochrein and Gros the following measurements may be added, in addition:

During asphyxial rise of pressures:

Aortic pressures: 80/56 100/76 130/106 142/113 104/85

Coronary pressures: 82/56 102/76 128/103 139/110 102/82

Before and after small intravenous doses of synephrine:

Aortic pressures: 78/58 109/90 125/104 148/121

Coronary pressures: 83/61 114/94 128/98 151/124

During depressor effect following central vagus stimulation:

Aortic pressures: 132/105 118/96 90/65 70/45

Coronary pressures: 132/105 118/95 88/64 72/44

Finally, in segment F, are shown end-pressure variations during successive cycles which vary in length as a result of a sinus arrhythmia. Con-

siderable variations in actual pressures obtain as a result. Again, a close and prompt correspondence occurs regardless of whether the curves are considered from the viewpoints of numerical systolic and diastolic readings or of detailed variations. The conception of Hochrein and Gros that rapid equilibration of pressures cannot occur is obviously erroneous.

SUMMARY

Pressure pulses were simultaneously recorded from the aorta and either from the central end or from a lateral branch of the anterior descending ramus of the left coronary artery of the dog. Calibrated optical manometers designed and described by one of us were used. They had a high figure of merit when tested under conditions of actual use. A technique was developed by which artefacts due to motions of the heart were eliminated.

Such simultaneous records give no indication that the pressure relations or form of the coronary pressure pulse are modified by any factor except the pressure changes in the aorta. The minor changes noted are such as occur in other branches of the aorta. The conclusions of Hochrein and Gros, that in addition heart rate, capacity and elasticity of the aorta, eddies and friction due to an unusually well developed intima are not supported by our studies. We regard these conclusions invalid for *a*, comparisons of coronary and carotid pulses do not serve for the study of the problem because the contour and pressure values of the latter differ essentially from those in the aorta, and *b*, evidence indicates that the records on which conclusions are based contain many artefacts and hence do not picture the correct form of pressure fluctuations either in the coronary or carotid arteries.

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A COMPARISON OF THE CLEARANCES OF CREATININE AND OF VARIOUS SUGARS

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The clearances of non-metabolized sugars have recently been proposed by Jolliffe, Shannon and Smith (1932) as a measure of glomerular filtration. They found almost completely identical clearances of xylose, of sucrose, of raffinose, and of glucose under phlorhizin. Shannon, Jolliffe and Smith (1932) found that creatinine clearance in the dog is 15 to 40 per cent greater than xylose, the extra creatinine being ascribed to tubular secretion. Under phlorhizin the creatinine clearance is the same as that of xylose and of glucose, which is interpreted as meaning that phlorhizin abolishes tubular secretion of creatinine. Earlier efforts by various workers to find two or more substances consistently showing the same clearance had been unsuccessful. The great importance of the question of the identity of clearances of two or more substances is obvious. We are reporting here a series of comparisons of the clearances of xylose, sucrose, glucose and creatinine, with and without phlorhizin, in both anesthetized and unanesthetized dogs.

METHODS. The experiments of table 1 were on dogs anesthetized by an intravenous injection of 0.25 gram sodium barbital per kilo. The ureters were cannulated and blood drawn from the femoral artery. In all other experiments unanesthetized female dogs were used; they were trained to lie quietly while blood was taken from a leg vein and urine by bladder catheterization. The catheterization was greatly facilitated by a mid-line episiotomy performed two or three weeks before beginning the experiments. The dogs were on a mixed diet of meat and table scraps; they were not fed on the morning of an experiment. During the experiment they were given the freedom of the room between collections and were given water *ad lib*, usually drinking a few hundred cubic centimeters. In all phlorhizin experiments without anesthetic (except expts. 16, 17 and 25) 0.2 gram of phlorhizin per kilo was given subcutaneously from 60 to 80 minutes before beginning the first collection period. In experiments 16, 17 and 25, in addition to this dose another 0.2 gram per kilo was given intravenously. Merck's phlorhizin purified according to Deuel and Chambers (1925) was

TABLE 1

Comparison of clearances in anesthetized dogs

Dog 1 was sick and in very poor condition. Mean blood pressure at end of experiments 3 and 5 was 124 and 110 mm. Hg, respectively. In experiments 1, 2 and 3, 0.2 gram phlorizin per kilo was given subcutaneously about 1½ hours before the first collection period and another 0.1 gram per kilo near the end of the first period; in experiment 5 no phlorizin was given. The dose of xylose and of sucrose was 2 grams per kilo subcutaneously about 2 hours before the first period; at the same time 0.1 gram creatinine per kilo was given in experiment 1, 0.15 gram in experiments 2 and 3, and 0.2 gram in experiments 4 and 5.

NUMBER, DATE, WEIGHT, (KILOS)	XYLOSE		SUCROSE		GLUCOSE		CREATININE		CLEARANCE = $\frac{UV}{N}$				CLEARANCE RATIOS		
	URINE VOLUME V														
	cc. per minute	mgm. per cent	Urine	Serum	Urine	Serum	Urine	Serum	Xylose cc. per minute	Sucrose cc. per minute	Glucose cc. per minute	Creati- nine cc. per minute	Xylose Creatinine	Sucrose Creatinine	Glucose Creatinine
1 2/17/33 7.7 kilos	0.220	6.010	107	107	5.010	71.3	263	3.3		12.4	15.4	17.5		0.71	0.88
	0.110	3.810	151		3.980	98.5	192	4.6		2.8	4.4	4.6		0.61	0.96
2 2/22/33 13.4 kilos	0.926	6.270	179		3.380	113	353	8.6		32.4	27.7	38.0		0.85	0.73
	0.670	5.920	158		3.820	130	369	8.1		25.1	19.7	30.6		0.82	0.64
	0.527	5.220	149		3.820	125	353	8.4		20.5	17.9	24.6		0.83	0.73
3 3/1/33 13.7 kilos	0.480	5.320	143		3.600	126	350	8.9		17.9	13.7	18.9		0.95	0.72
		6.980	116		6.400	133	515	7.6		52.0	41.6	58.6		0.89	0.71
		7.200	94.1		7.150	145	498	7.1		61.7	39.8	56.6		1.09	0.70
5 3/8/33 15.0 kilos		6.010	87.1		7.440	145	464	7.0		55.2	41.0	52.9		1.04	0.78
		5.790	67.2		8.190	143	457	6.0		58.1	38.6	51.4		1.13	0.75
	0.790	4.330	105		4.87	223	615	10.8		32.6	47.2	45.0		1.05	0.68
	0.683	4.550	97.1		5.10	266	632	10.2		32.1	39.3	42.4		0.76	0.93
	0.620	4.310	88.2		7.36	301	609	9.5		30.3	35.5	39.7		0.90	0.68
	0.500	4.160	78.7		8.18	333	632	9.1		26.5	28.2	34.7		0.81	0.63
	0.353	4.030	73.9		1.156	355	632	8.9		19.3	18.0	25.1		0.77	0.72

TABLE 2
Comparison of xylose, sucrose, glucose and creatinine clearances in phlorhizinized dogs

NUMBER DATE	URINE VOLUME V	XYLOSE		SUCROSE		GLUCOSE		CREATININE		CLEARANCE = $\frac{UV}{Sv}$				CLEARANCE RATIOS				
										Xylose	Glucose	Creatinine	Xylose	Glucose	Creatinine	Xylose	Glucose	Xylose
		Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum									
	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute
6 3/13/33 Dog 14.8 kilos	1.69	2,450	113	2,530	119	2,780	105	642	21.1	36.9	36.0	44.8	51.4	0.72	0.70	0.81	0.98	1.21
	1.51	2,460	116	2,770	133	2,690	105	494	17.7	32.2	31.4	38.2	42.1	0.76	0.75	0.91	0.97	1.19
	1.61	2,420	119	2,930	140	2,930	140	400	15.9	32.7	33.0	41.8	40.4	0.81	0.83	1.04	1.03	1.28
	1.74	2,460	112	2,750	133	2,750	133	383	15.9	32.7	33.0	41.8	40.4	0.81	0.80	1.03	0.98	1.33
	1.38	2,260	109	2,980	132	2,770	102	314	12.9	28.6	31.2	37.3	35.5	0.83	0.93	1.12	1.09	1.31
17 5/1/33 Dog 1	1.68	2,370	76.4	2,580	78.8	3,350	89.5	311	7.2	52.0	55.3	62.8	72.6	0.72	0.76	0.85	1.06	1.21
	1.71	2,440	88.4	2,800	94.5	3,150	86.0	319	8.6	47.2	50.6	62.6	63.4	0.74	0.80	0.99	1.07	1.36
	1.79	2,540	91.4	2,970	107	3,010	87.0	323	9.1	49.8	49.8	62.0	63.6	0.78	0.78	0.98	1.00	1.25
	1.79	2,400	91.4	2,940	106	2,990	74.3	332	9.7	47.1	49.8	71.9	61.2	0.77	0.81	1.17	1.06	1.53
	1.71	2,370	85.6	2,900	91.1	3,030	82.3	337	9.7	47.4	54.4	62.9	59.4	0.80	0.92	1.06	1.15	1.33
12 4/6/33 Dog 1	2.08	2,540	157	2,860	144	2,100	112	326	15.4	33.7	41.4	38.9	44.1	0.76	0.94	0.88	1.23	1.15
	2.09	2,600	131	2,730	143	2,470	105	347	14.3	40.6	56.6	58.6	39.8	0.82	0.91	0.97	1.28	1.25
	1.84	2,490	131	3,150	129	2,730	105	347	14.3	40.6	56.6	58.6	39.8	0.82	0.91	0.97	1.28	1.25
	1.84	2,490	131	3,150	129	2,730	105	347	14.3	40.6	56.6	58.6	39.8	0.82	0.91	0.97	1.28	1.25
	1.45	2,280	95.8	2,980	101	3,350	111	337	13.2	34.5	42.8	43.8	37.0	0.83	1.16	1.18	1.24	1.27
16 4/26/33 Dog 1	2.80	2,200	117	1,990	124	1,770	119	294	11.6	52.6	45.1	41.7	70.8	0.75	0.64	0.59	0.86	0.79
	2.44	2,430	123	2,680	127	2,450	119	337	12.4	48.3	51.5	50.2	66.4	0.73	0.78	0.76	1.07	1.04
	1.97	2,660	123	3,070	134	2,860	115	366	12.8	42.6	45.1	49.0	56.4	0.76	0.80	0.92	1.06	1.15
	1.89	2,610	117	3,180	140	3,210	115	370	12.6	42.2	42.9	52.7	55.6	0.76	0.77	0.95	1.02	1.24
	1.68	2,380	103	3,250	121	3,370	116	366	11.7	38.8	45.2	48.7	52.6	0.74	0.86	0.93	1.17	1.26
8 3/20/33 Dog 2 11 kilos	0.820	2,380	79.2	2,630	92.8	3,260	102	314	8.6	24.9	23.5	26.6	27.7	0.82	0.78	0.96	0.94	1.07
	0.953	2,340	88.1	2,900	107	3,090	99.9	335	9.4	25.3	25.8	29.6	30.9	0.74	0.76	0.96	1.02	1.17
	0.923	2,370	96.6	3,040	120	3,090	96.3	343	11.0	22.6	23.3	29.9	31.0	0.78	0.81	0.95	1.03	1.31
	1.17	2,330	96.9	3,040	123	3,140	114	343	13.2	28.2	28.2	29.8	32.5	0.83	0.95	0.96	1.02	1.15
	0.987	2,240	101	3,030	112	3,230	120	353	11.7	21.9	26.8	26.5	27.8	0.74	0.90	0.95	1.22	1.21
12 4/3/33 Dog 2	1.28	2,480	115	2,590	118	2,760	105	328	9.8	27.0	28.0	33.7	42.9	0.64	0.65	0.79	1.01	1.22
	1.10	2,760	113	3,170	117	3,190	107	370	10.7	26.8	29.8	32.7	38.1	0.70	0.78	0.86	1.11	1.22
	1.05	2,750	108	3,170	115	3,320	117	382	10.7	26.8	29.8	31.6	37.5	0.71	0.77	0.84	1.08	1.18
	0.913	2,720	101	3,260	113	3,380	111	422	10.7	24.5	26.4	27.9	33.1	0.68	0.73	0.77	1.07	1.13
	0.930	2,540	89	3,080	110	3,500	109	412	11.0	25.5	26.0	30.0	34.9	0.76	0.75	0.86	0.98	1.13
14 4/8/33 Dog 2	1.98	2,620	157	3,390	164	2,260	120	308	16.4	31.7	41.0	37.2	37.2	0.85	1.00	1.00	1.29	1.17
	1.43	2,980	163	2,900	174	2,720	125	341	16.3	27.3	23.9	31.2	28.9	0.80	0.80	1.04	0.87	1.14
	1.35	2,800	140	3,620	186	2,750	122	330	15.4	27.0	26.2	30.4	28.9	0.94	0.91	1.05	0.97	1.13
	1.13	2,520	133	3,240	161	3,140	126	331	14.9	21.5	22.7	28.1	23.1	0.89	0.92	1.12	1.06	1.31

TABLE 3
Comparison of xylose, sucrose, glucose and creatinine clearances in non-phlorhizinized dogs

NUMBER DATE	URINE VOLUME v	XYLOSE		SUCROSE		GLUCOSE		CREATININE		CLEARANCE = $\frac{UV}{S}$				CLEARANCE RATIOS		
		Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum	Xylose	Sucrose	Glucose	Creati- nine	Creatinine	Sucrose	Xylose
	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute
7																
3/16/33	1.55	3.880	134	368	108	12.6	45.0	502	12.6	52.5	5.3	52.5	61.8	0.85	0.78	1.17
Dog 1	1.63	3.290	143	329	106	488	13.6	488	13.6	45.8	5.0	45.8	58.5	0.64	0.90	1.43
	1.41	3.070	147	322	110	446	14.3	446	14.3	42.2	4.2	42.2	46.8	0.63	0.83	1.29
	1.66	3.040	141	332	110	486	14.4	486	14.4	55.9	4.6	55.9	56.2	0.69	0.83	1.29
	1.34	3.290	154	176	102	155	13.2	155	13.2	35.5	3.9	35.5	54.0	0.66	0.72	1.10
9																
3/23/33	1.33	3.360	152	353	92.9	515	13.2	515	13.2	39.3	3.9	39.3	51.7	0.57	0.76	1.35
Dog 1	1.31	3.190	158	356	107	511	13.7	511	13.7	26.5	3.7	26.5	48.0	0.54	0.70	1.42
	1.22	3.160	154	274	93.0	511	13.4	511	13.4	25.0	3.2	25.0	46.5	0.54	0.70	1.30
	1.13	3.070	149	243	96.1	506	13.2	506	13.2	23.3	2.8	23.3	43.3	0.54	0.77	1.44
18																
5/12/33	1.02	4.050	119	430	99.3	541	9.1	541	9.1	34.7	4.4	34.7	60.7	0.57	0.77	1.35
Dog 1	1.09	4.090	123	365	103	569	9.6	569	9.6	36.3	3.8	36.3	64.6	0.56	0.77	1.38
	1.07	4.180	117	535	106	663	9.3	663	9.3	38.2	4.9	38.2	69.4	0.55	0.72	1.31
	0.97	4.500	105	475	111	660	8.9	660	8.9	41.5	5.1	41.5	72.0	0.58	0.72	1.24
	0.82	4.860	90.8	514	110	715	7.8	715	7.8	43.9	3.9	43.9	75.2	0.58	0.66	1.14
10																
3/27/33	0.787	3.270	94.6	428	96.1	474	7.5	474	7.5	27.3	3.5	27.3	49.8	0.55	0.71	1.29
Dog 2	0.743	3.470	104	448	93.2	506	8.4	506	8.4	24.8	2.9	24.8	44.1	0.55	0.67	1.20
	0.747	3.790	105	454	96.5	543	9.2	543	9.2	27.0	3.6	27.0	44.1	0.61	0.82	1.34
	0.663	3.940	89.8	545	110	572	8.5	572	8.5	29.1	3.2	29.1	44.6	0.63	0.72	1.11
	0.753	4.050	96.5	461	102	600	9.0	600	9.0	31.6	3.4	31.6	50.2	0.63	0.84	1.33
22																
5/24/33	0.667	4.110	87.9	441	106	702	9.0	702	9.0	31.2	3.3	31.2	52.0	0.60	0.76	1.26
Dog 4	0.633	4.790	100	515	101	825	10.2	825	10.2	30.3	3.6	30.3	51.3	0.59	0.70	1.19
13.0 kilos	0.813	3.830	110	449	104	714	11.1	714	11.1	28.2	3.1	28.2	52.3	0.54	0.71	1.12
	0.710	4.410	110	497	110	859	11.6	859	11.6	28.5	3.3	28.5	49.4	0.58	0.68	1.17
23																
5/29/33	0.953	4.120	116	212	118	816	14.8	816	14.8	33.8	4.1	33.8	52.6	0.64	0.78	1.22
Dog 1	1.04	3.860	121	298	105	780	16.0	780	16.0	33.2	3.7	33.2	50.8	0.65	0.66	1.02
	1.05	4.060	116	213	110	838	15.6	838	15.6	36.7	4.3	36.7	56.4	0.65	0.80	1.23
	0.873	4.340	100	258	117	915	13.8	915	13.8	37.9	4.2	37.9	58.0	0.65	0.81	1.25
24																
6/2/33	0.447	6.810	83.1	40	100	1,390	10.7	1,390	10.7	36.6	0.2	36.6	58.2	0.63	0.83	1.26
Dog 4	0.470	6.520	80.6	105	106	1,380	11.2	1,380	11.2	38.0	0.4	38.0	57.8	0.66	0.66	1.02
	0.430	6.670	81.2	163	103	1,460	11.6	1,460	11.6	35.0	0.7	35.0	54.2	0.60	0.77	1.28
	0.387	7.380	76.4	201	90.5	1,680	11.4	1,680	11.4	37.7	0.9	37.7	59.9	0.60	0.80	1.33
	0.380	7.440	75.5	101	91.3	1,730	11.4	1,730	11.4	37.4	0.4	37.4	57.8	0.63	0.81	1.25

used in all experiments. Urine collection periods were in all cases 30 minutes with a blood sample at the middle of each period. All blood samples were centrifuged immediately after collection; the iron filtrates of both serum and urine were made on the day of the experiment. The dose of xylose was 2 grams, of sucrose 2 grams and of creatinine 0.2 or 0.3 gram per kilo, given subcutaneously about 90 minutes before the first period. The chemical methods are discussed in the appendix. In table 2 are seen the results where the clearances of xylose, sucrose, glucose under phlor-

TABLE 4
Comparison of sucrose, glucose and creatinine clearances in phlorhizinized dogs

NUMBER DATE	URINE VOLUME V cc. per minute	SUCROSE		GLUCOSE		CREATININE		CLEARANCE = $\frac{UV}{Se}$			CLEARANCE RATIOS	
		Urine mgm. per cent	Serum mgm. per cent	Urine mgm. per cent	Serum mgm. per cent	Urine mgm. per cent	Serum mgm. per cent	Sucrose cc. per min- ute	Glucose cc. per min- ute	Creatinine cc. per min- ute	Sucrose Creatinine	Glucose Creatinine
4 3/4/33 Dog 1	1.30	5,220	178	2,790	101	482	15.5	38.1	35.9	40.4	0.94	0.89
	1.60	5,520	171	3,130	104	510	15.1	51.8	48.2	54.2	0.95	0.89
	1.25	5,590	154	3,550	100	533	14.2	45.4	44.4	47.0	0.96	0.94
	1.25	5,350	122	3,880	96.0	524	12.6	54.6	50.5	51.9	1.05	0.97
	1.11	4,960	105	4,320	100	551	11.2	52.4	47.9	54.6	0.96	0.86
11 3/30/33 Dog 1	1.97	6,270	205	3,170	90.4	615	15.3	60.3	69.2	79.2	0.76	0.87
	1.52	6,200	187	3,640	95.6	591	14.5	50.5	58.0	61.9	0.82	0.94
	1.46	5,890	152	4,020	91.3	591	12.7	56.5	64.5	68.0	0.83	0.95
	1.08	5,380	119	4,310	92.9	586	10.2	48.8	50.1	62.0	0.79	0.81
	1.13	5,020	93.3	4,910	89.0	588	8.8	60.8	62.4	75.5	0.80	0.83
19 5/15/33 Dog 3 8.7 kilos	0.537	3,660	76.5	5,310	86.5	561	8.2	25.2	33.0	36.7	0.69	0.90
	0.623	4,320	90.5	5,020	88.8	628	9.8	29.7	35.3	39.9	0.74	0.88
	0.613	5,140	96.0	5,020	82.7	674	10.6	32.8	37.2	39.0	0.84	0.95
	0.640	4,660	88.8	5,200	86.2	682	10.7	33.6	38.6	40.8	0.82	0.95
	0.590	4,240	82.5	5,220	86.2	698	10.3	30.3	35.7	40.0	0.76	0.89

hizin and creatinine were determined simultaneously, in table 3 the comparisons of xylose, sucrose and creatinine without phlorhizin (sucrose omitted in expt. 24), in table 4 the comparisons of sucrose, glucose and creatinine and in table 5 xylose, glucose and creatinine.

DISCUSSION. Table 1 shows that with anesthetized dogs the sucrose clearance is usually not greatly different from the creatinine, although differences of as much as 25 per cent may exist. The relation is not influenced by phlorhizin. Creatinine clearance definitely exceeds glucose even under phlorhizin. Xylose clearance is less than either sucrose or

creatinine. The fall in clearances sometimes seen toward the end of an experiment is interpreted as due to a beginning renal circulatory deficit. It is felt that the anesthetic may differentially alter the permeability of the renal tubules to the various constituents so as to change the ratio of their clearances from the normal state. More importance is, therefore, attached to the experiments on unanesthetized dogs. We find (table 3) that sucrose clearance is always significantly higher than xylose in the absence of phlorhizin, creatinine being higher than either. Under phlorhizin (table 2) the xylose clearance approaches that of sucrose, although it does not always reach it. We fail to find the xylose clearance equaling that of creatinine under phlorhizin, as did Shannon, Jolliffe and

TABLE 5
Comparison of xylose, glucose and creatinine clearances in phlorhizinized dogs

NUMBER DATE	URINE VOLUME V cc. per minute	XYLOSE		GLUCOSE		CREATININE		CLEARANCE = $\frac{UV}{Se}$			CLEARANCE RATIOS	
		Urine	Serum	Urine	Serum	Urine	Serum	Xylose	Glucose	Creatinine	Xylose Creatinine	Glucose Creatinine
20 5/18/33 Dog 1	1.49	3,710	115	3,250	87.4	444	10.2	48.1	55.4	64.8	0.74	0.85
	1.58	3,630	116	3,440	92.4	444	10.3	49.4	58.9	68.1	0.73	0.86
	1.51	3,690	102	3,890	99.2	465	9.5	54.6	59.2	74.0	0.73	0.80
	1.41	3,620	92.5	4,210	90.7	488	9.2	55.1	65.4	74.8	0.74	0.87
	1.30	3,460	82.1	4,460	90.1	484	8.7	54.7	64.2	72.3	0.76	0.89
21 5/22/33 Dog 3	0.820	3,380	121	3,600	104	522	13.5	22.9	28.4	31.7	0.72	0.90
	0.903	3,600	129	3,310	95.9	562	14.8	25.2	31.1	34.3	0.73	0.91
	0.923	3,500	124	3,470	98.5	558	14.4	26.0	32.6	35.7	0.73	0.91
	0.907	3,360	106	3,960	96.4	583	13.5	28.8	37.3	36.5	0.79	1.02
	0.850	3,260	94.5	4,410	88.1	592	12.3	29.3	42.5	40.9	0.72	1.04

Smith, although the difference is less with phlorhizin than without. This might mean either that phlorhizin inhibits the tubular secretion of creatinine, as suggested by Shannon, Jolliffe and Smith, or the reabsorption of xylose.

In an effort to decide between these two possibilities we have taken the averages of the creatinine, sucrose and xylose clearances with and without phlorhizin in all the experiments on dog 1 and dog 2. On dog 1 the average creatinine clearance without phlorhizin (23 periods) was 56.1 cc. per minute, with phlorhizin (35 periods) 56.9 cc. The sucrose clearance without phlorhizin (19 periods) was 44.0 cc. and with phlorhizin (30 periods) 46.3 cc. The xylose clearance without phlorhizin (18 periods) was 34.8 cc., with

TABLE 6
Effect of phlorhizin on xylose, sucrose, glucose and creatinine clearances

NUMBER DATE	URINE VOLUME v	XYLOSE		SUCROSE		GLUCOSE		CREATININE		CLEARANCE = $\frac{UV}{S}$				CLEARANCE RATIOS															
		Urine		Serum		Urine		Serum		Urine		Serum		Xylose		Sucrose		Glucose		Creatinine		Xylose		Glucose		Creatinine		Xylose	
		mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	
25 6/10/33 Dog 1	cc. per minute																												
	0.797	5,790	103	109	94.3	1,120	14.2	44.8	1.0	62.9	0.71																		
	0.957	5,380	119	143	88.8	1,040	16.8	43.2	1.5	59.2	0.73																		
	0.857	5,660	126	198	87.0	1,160	17.5	38.6	2.0	56.8	0.68																		
Phlorhizin injected: 0.2 gram per kilo subcutaneously and 0.2 gram per kilo intravenously, 30 minute interval after injections																													
26 6/14/33 Dog 4	1.36	3,630	116		3,380	90.0	656	18.3	42.6		51.1	48.7	0.87																
	1.13	3,680	110		3,490	87.2	718	17.7	37.8		45.2	45.9	0.82																
	1.13	3,460	102		3,620	89.9	718	16.9	38.3		45.6	48.0	0.80																
	1.29	3,080	119	3,460	105	160	112	609	14.8	33.4	42.6	1.8	53.2	0.63	0.80														
	1.24	3,230	115	3,810	102	289	110	659	14.3	34.8	46.2	3.2	57.2	0.61	0.81														
	Phlorhizin injected: 0.2 gram per kilo subcutaneously, 60 minute interval after injection																												
	1.80	2,210	84.5	2,480	94.8	3,360	110	454	13.2	47.2	47.2	54.9	61.9	0.76	0.76	0.89	1.00	1.16											
	1.50	2,090	78.7	2,340	85.6	3,500	98.2	456	12.7	39.9	41.0	53.4	53.8	0.74	0.76	0.99	1.03	1.34											
1.42	1,970	71.6	2,360	81.0	3,580	97.0	462	12.3	39.1	41.3	52.4	53.4	0.73	0.77	0.98	1.06	1.34												
1.39	1,880	64.5	2,190	76.1	3,780	94.7	458	11.9	40.6	40.0	55.4	53.6	0.76	0.75	1.03	0.99	1.37												

phlorhizin (25 periods) 43.0 cc. It thus appears that creatinine clearance is unchanged by phlorhizin, xylose significantly increased and sucrose unchanged or slightly increased. Since glucose clearance under phlorhizin is in most cases essentially the same as that of creatinine we must assume that there is no tubular secretion of creatinine under phlorhizin. Since, furthermore, creatinine clearance without phlorhizin is no greater than with phlorhizin it follows that there is no tubular secretion of creatinine without phlorhizin. The greater xylose clearance with than without phlorhizin is explained on the basis that there is a significant reabsorption of xylose without phlorhizin; this is diminished by phlorhizin but is not totally abolished, as evidenced by the fact that xylose clearance with phlorhizin is still somewhat less than creatinine. Sucrose clearance without phlorhizin is less than creatinine and more than xylose, which means that there is some reabsorption of sucrose, although less than of xylose. With phlorhizin sucrose clearance rises very slightly but is still less than creatinine, which is interpreted as meaning that tubular reabsorption of sucrose is but little influenced by phlorhizin. Further evidence (expt. 26, table 6) on this point was obtained by comparing creatinine, sucrose, xylose and glucose clearances in a single experiment before and after phlorhizin. It is seen that creatinine and sucrose clearances are unchanged by phlorhizin, while xylose is definitely increased, the interpretation being as above.

This is not an invariable behavior; i.e., we may see creatinine clearance diminished and xylose unchanged by phlorhizin, as reported by Shannon, Jolliffe and Smith. Thus, in the averages of all the experiments on dog 2 we find creatinine clearance (5 periods) without phlorhizin 46.8 cc. and with phlorhizin (14 periods) 33.1 cc., sucrose (5 periods) without phlorhizin 35.0 cc. and with phlorhizin (14 periods) 27.2 cc., and xylose (5 periods) without phlorhizin 24.6 cc. and with phlorhizin (14 periods) 25.9 cc. Our interpretation of these results is that, on the average, the renal circulation of dog 2 was depressed by phlorhizin to the extent that the glomerular filtration was diminished as shown by the creatinine clearances. The xylose clearance was not diminished because the diminution in tubular reabsorption of xylose about balanced the diminished glomerular filtration. The sucrose clearance was diminished more than the xylose and almost as much as the creatinine, because sucrose reabsorption is but little influenced by phlorhizin. Experiment 25 in table 6 is given the same interpretation. This experiment was on dog 1 which, on the average, showed no decrease in creatinine and a rise in xylose clearance with phlorhizin. It thus appears that a given dog may give the results of either the present or the preceding paragraph, presumably depending upon the effect of the phlorhizin on the glomerular circulation. Such a depressing effect might be expected to be greater with an intravenous than with a

subcutaneous dose. This dose of phlorhizin intravenously always produces vomiting, subcutaneously never. It may be pointed out that our interpretation is consistent with either result, while that of Shannon, Jolliffe and Smith fails to explain the large number of cases where creatinine clearance is unchanged and xylose clearance is increased by phlorhizin.

Under phlorhizin both xylose and sucrose clearances are almost always less than glucose, which in a little more than half the cases is the same (within 10 per cent) as creatinine. Shannon, Jolliffe and Smith found that glucose and creatinine clearances are equal under phlorhizin. We reported recently that on anesthetized dogs (White and Monaghan, 1933) the creatinine clearance practically always significantly exceeded the glucose under phlorhizin and this is still found in the anesthetized series (table 1) of the present paper. With unanesthetized dogs, however, we are nearer to Shannon, Jolliffe and Smith's finding of an identity of glucose and creatinine clearances under phlorhizin, although our findings are not so consistent as theirs.

It will be noted that there are usually a few hundred milligrams of glucose per 100 cc. of urine after the subcutaneous dose of sugars plus creatinine without phlorhizin. This is higher than the normal glucose content (25 to 30 mgm. per 100 cc.) of dog urine found by West, Lange and Peterson (1932). Whether this is due in some way to the action of a hypertonic solution subcutaneously injected, to the diuresis alone or to some other factor cannot be answered at present. It is possible that some hydrolysis of sucrose takes place on subcutaneous injection even though none has been reported on intravenous injection (Keith, Wakefield and Power, 1932). Part of the apparent glucose might thus be fructose; however, this factor, if present at all, is not the only one since the glucose content of the urine may be as high with xylose alone as with xylose plus sucrose. The matter demands further investigation but its answer would have no significant bearing on our present problem, since at the most it could introduce an error of only a few per cent in the urine sucrose and glucose figures.

It is our present feeling that the clearance of glucose under phlorhizin or of creatinine with or without phlorhizin comes closer to representing glomerular filtration than does any other substance so far proposed. We cannot find as close an agreement between these clearances and those of xylose and sucrose as did Jolliffe, Shannon and Smith. We believe, on the basis of many determinations on known mixtures of sugars and creatinine and their recoveries from serum and urine that our analytical figures have a maximum error of 5 per cent, with usually not more than 3 per cent. Whether the discrepancies between their findings and ours are entirely a matter of chemical methods cannot be answered with certainty. We have exchanged views with the above workers and some of the apparent

discrepancies have found an explanation but on certain points we are still at variance. These may be briefly summarized as follows. Whereas Jolliffe, Shannon and Smith find practically complete identity of the clearances of xylose and of sucrose without phlorhizin and of xylose, sucrose, glucose and creatinine with phlorhizin, we find sucrose clearance 15 to 45 per cent higher than xylose without phlorhizin; sucrose and xylose are nearer together with phlorhizin than without but both definitely less than glucose or creatinine. We confirm their finding that without phlorhizin creatinine clearance is greater than either sucrose or xylose but differ both in findings and interpretation on the effect of phlorhizin upon creatinine and xylose clearances.

We are inclined to agree with Poulsson (1930) that the lower glucose than creatinine clearance found by him on unanesthetized dogs under phlorhizin was due largely to inadequate chemical methods. If our interpretation of the results reported here is correct the original view of Rehberg (1926) that creatinine clearance with an artificially raised plasma creatinine level offers a practical means of estimating glomerular filtration is supported. We believe, however, that the normal plasma content of true creatinine is so low that its urinary output cannot be affected by glomerular filtration alone. It may appear illogical to hold that the tubules can secrete creatinine from a normally existing creatinine precursor and cannot secrete artificially introduced preformed creatinine. We prefer not to be dogmatic on this point but merely to state that this view seems at present to offer the least difficulty in an interpretation of our findings. If we are correct in our opinion that creatinine or glucose clearance affords a reasonably accurate estimate of glomerular filtration rate it follows that the elimination of all other normal urinary constituents (with certain exceptions, as ammonia and hippuric acid) in the dog can be accounted for by filtration alone.

SUMMARY

1. In unanesthetized dogs without phlorhizin the clearance of xylose is definitely less than that of sucrose, which is definitely less than that of creatinine.
2. With phlorhizin the xylose clearance approaches that of sucrose, both being less than creatinine or glucose, which in more than half the cases are essentially the same.
3. Creatinine clearance may or may not be diminished by phlorhizin; xylose clearance may rise or remain unchanged. Evidence is presented that these findings may be better interpreted as an effect of phlorhizin on glomerular circulation and on tubular reabsorption of xylose than as an effect on tubular secretion of creatinine.
4. It is believed that the clearance either of glucose under phlorhizin

or of creatinine represents a better measure of glomerular filtration than does either sucrose or xylose, which give figures from 15 to 45 per cent too low.

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APPENDIX. Serum and urine filtrates. All sugar determinations were carried out on a modification of the iron filtrate described by Steiner, Urban and West (1932).

Serum. To 2 volumes of serum in an Erlenmeyer flask are added 1 volume of 12 per cent $\text{Fe}_2(\text{SO}_4)_3$ and 7 volumes of water. After mixing, 1 gram of BaCO_3 for each cubic centimeter of iron solution is added, the mixture shaken vigorously until no more gas is evolved, and filtered. The filtrate contains an excess of barium which may be precipitated by the addition of a small amount of Na_2SO_4 (2 to 3 mgm. per cc. of filtrate). The precipitate is centrifuged or filtered off.

Urine. One cubic centimeter of urine is transferred to a 50 cc. volumetric flask, 10 cc. of 12 per cent $\text{Fe}_2(\text{SO}_4)_3$ are added and the flask made up to volume with distilled water. The contents are transferred to an Erlenmeyer flask containing 12 grams BaCO_3 , shaken, filtered, and the excess barium removed as above. Suitable dilutions are prepared for sugar analyses.

Xylose. Sucrose and glucose are removed from filtrates containing all three sugars by yeast fermentation. A 20 per cent suspension of Fleischman's yeast, washed repeatedly until the supernatant liquid is clear and free from reducing substances, is used. Two cubic centimeter portions of this suspension are transferred to centrifuge tubes and centrifuged for 10 minutes. The supernatant fluid is decanted, the tubes inverted and allowed to drain for 10 minutes. Excess water is removed from the sides with filter paper. Six cubic centimeters of blood or urine filtrate, containing not more than 2 mgm. of sucrose and glucose combined, are transferred to each tube and stirred with the yeast. Fermentation is continued for half an hour in a water bath at 38° . The yeast is then centrifuged down and the supernatant fluid filtered.

Xylose determinations are carried out on 5 cc. of filtrate, using copper reagent no. 50 of Shaffer and Somogyi (1933), with 200 cc. of 0.1 N KIO_3 and 1 gram KI per liter. The tubes are heated for 15 minutes; 98 per cent of the maximum reduction is obtained in that time. Since the reduction is not strictly proportional to the amount of sugar present, a curve was prepared from analyses of known xylose solutions and the factor read from the curve. Between 1 and 2 per cent (average 1.6 per cent) of the xylose disappears during yeast treatment, but since the percentage is the same in serum and urine filtrates the concentration ratios are unaffected and hence the xylose figures are not corrected for this loss. A correction was made, however, for non-fermentable reducing substances other than xylose which amounted on the average to the equivalent of 2 mgm. xylose per 100 cc. for serum and 160 mgm. per 100 cc. for urine under the conditions of these experiments.

Glucose. The difference between the titrations of the fermented and the unfermented unhydrolyzed filtrates represents glucose. The presence of xylose does not interfere with the reducing power of the glucose, but the factor used in the calculation depends upon the total amount of reducing substances present, so that if the mixture contains 100 mgm. per cent each of glucose and xylose the amount of glucose is obtained by multiplying the difference between fermented and unfermented unhydrolyzed titrations by a factor corresponding to 200 mgm. per cent of glucose, a correction must then be applied for the xylose which disappears into the yeast; i.e.,

1.6 per cent of the xylose figure is subtracted from the glucose value obtained on the same filtrate.

Since the blood used in these analyses was drawn from a leg vein (the common stem of the dorsal digital veins), whereas the blood in the glomeruli is arterial, several comparisons were made of the glucose content of venous and arterial blood drawn in rapid succession from the leg vein and the femoral artery, respectively, of unanesthetized phlorhizinized dogs. The arterial blood contained from 2 to 7 per cent (average 5 per cent) more glucose than the venous blood. A 5 per cent correction was, therefore, added to all serum glucose figures. Xylose, sucrose and creatinine concentrations were found to be practically identical in venous and arterial blood.

Sucrose. The difference between the titrations of hydrolyzed and unhydrolyzed samples of the same filtrate represents sucrose. The hydrolysis is carried out as follows: To 10 cc. of filtrate in a test tube is added 1 cc. of $N/2 H_2SO_4$. The tubes are covered with glass bulbs, placed in a boiling water bath for 5 minutes, cooled, and neutralized with 1 cc. of $N/2 NaOH$. The sugar analysis is carried out on an aliquot portion. A curve previously constructed from analyses of known sucrose solutions was used in the calculations, and as in the case of glucose, the factor selected from the curve is that corresponding to the total reduction obtained, whether the reduction is due to sucrose alone or to mixtures of two or more sugars.

In a number of determinations on known mixtures of the three sugars, and on blood and urine samples to which known amounts of xylose and sucrose had been added, each sugar was recovered with an error of less than 5 per cent by the methods described above.

Creatinine. Serum. The iron filtrates described above were found to be suitable for serum creatinine determinations. To 5 cc. of filtrate in a test tube are added 2 cc. of alkaline picrate (1 part of 10 per cent $NaOH$ to 5 parts saturated picric acid, purified according to Folin and Doisy, 1917). After 10 minutes the unknowns are compared in a compensating colorimeter with appropriate standards similarly treated.

Urine. Since part of the creatinine in the urine is precipitated by the iron treatment, creatinine determinations were carried out on untreated urine according to Folin (1914).

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EXPERIMENTAL CONSIDERATION OF VERTEBRAL ARTERY-CAROTID ARTERY ANASTOMOSES

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Since the discovery of carotid sinus (and body) reflexes, papers have appeared in the literature in which simple occlusion of the common carotids has been assumed to eliminate any possible rôle of carotid reflexes in responses to chemicals or drugs injected into the general circulation. In view of such papers and the possible appearance of others in the future, it has seemed justifiable to present observations emphasizing the functional significance of very free anastomoses between the vertebral artery and the carotid artery (Ellenberger and Baum, 1891). Because of such anastomoses the carotid body appears in many cases to be as freely reached by arterial blood with the common carotids occluded as with them patent.

A consideration of these observations at the same time answers the question brought up in a preliminary report (Winder, Owen and Gesell, 1932) as to whether a certain abrupt, intense, reflex-like respiratory response, obtained by injection of small doses of sodium cyanide into the vertebral artery while the carotids were occluded, is due to "an action of cyanide at the center, as an augmentation of peripheral effects, possibly from the non-pulsating or less turgid sinus," or to "some remarkably freely attained reflex zone innervated through the carotid plexus."

It was indicated in a previous paper (Winder and Winder, 1933) that the intracranial effects of sulfide on pulmonary ventilation, elicited by vertebral intra-arterial injections, did not differ essentially when the carotid sinus region was denervated or normally innervated, provided that in the latter case care was taken not to interfere in any way with the carotid blood flow. Respiratory responses to vertebral artery injections with the sinuses innervated showed nothing of the abrupt carotid reflex effect elicitable by sulfide in the carotid sinus (Heymans, Bouckaert and Dautrebande, 1931a, 1931b; Owen and Gesell, 1931; Winder and Winder, 1933) or carotid body (Dautrebande, 1931). This is evidence that with proper precautions and reservations the central effect of a chemical can be studied in the normally innervated animal by means of vertebral injections. That is, employing the precaution mentioned, a chemical injected into the vertebral artery of a dog usually does not come into relationship with the carotid sinus nerve terminations through the free vertebral-carotid anastomoses.

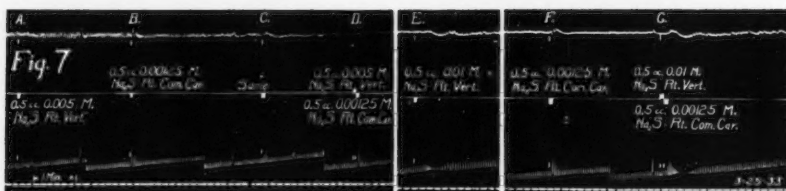
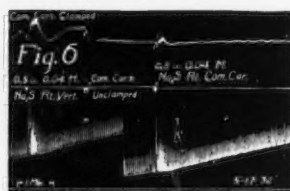
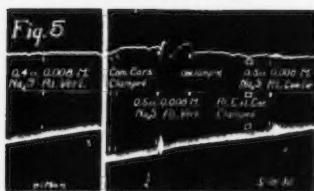
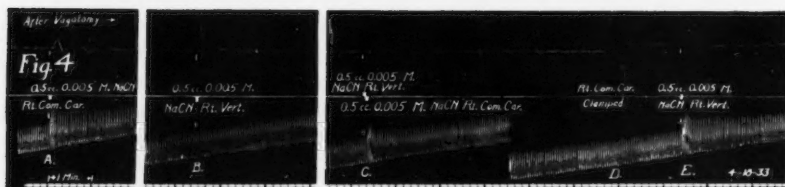
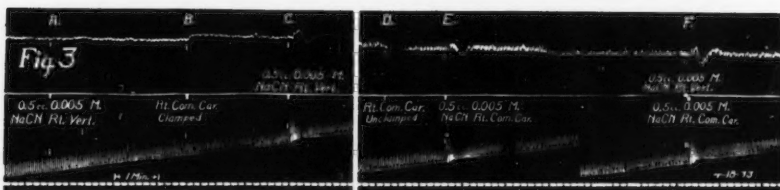
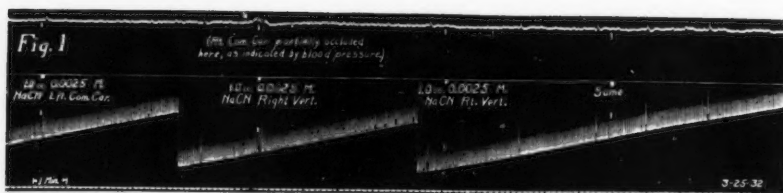
This had been determined at the time of reporting on the seat of action of *cyanide* on pulmonary ventilation (Winder, Winder and Gesell, 1933). However, it would have been somewhat more hazardous, in the case of that chemical, to assure one's self by observation of records from the normally innervated animal that the vertebral responses were free from carotid reflexes elicitable by the cyanide (Heymans, Bouckaert and Dautrebande, 1931b, 1931d; Owen and Gesell, 1931; Winder, Winder and Gesell, 1933) because the response to vertebral artery injection of cyanide after carotid denervation is usually characterized by some sort of stimulation. Consequently, in that paper, those vertebral responses elicited only after double carotid sinus denervation were described.

However, as briefly mentioned in the preliminary report (Winder, Owen and Gesell, 1932), many vertebral artery injections of cyanide, as of sulfide, have been made before denervation. As was the case for sulfide, the respiratory responses before and after denervation were able in general to be described and classified similarly, provided that care was taken not to obstruct the carotid flow.

The work of Gay (1930) indicates that cyanide and sulfide (as also oxygen deficiency) augment the efficiency of reflex myo-neural mechanisms. If such an augmentative influence be assumed for carotid sinus impulses entering the respiratory mechanism, and if carotid impulses are tonic, the apparent similarity of results from vertebral injections before and after sinus denervation would need to be explained by mutually cancelling influences on both inhibitory and excitatory impulses (Danielopolu et al.; 1930, 1931, 1932) by the chemical.

The question naturally arises as to how respiratory responses to vertebral artery injections of cyanide or sulfide may be altered by changing the balance of impulses from the carotid sinus region. The most obvious means of altering such impulses is by alterations of the blood pressure in the carotid system. Heymans and others (Heymans and Bouckaert, 1930a; Schmidt, 1932) have found that increased carotid pressure reflexly decreases pulmonary ventilation, whereas decreased pressure increases it.

We have frequently observed that an inadvertent partial occlusion of the common carotid (hence reduction in carotid pressure), during vertebral artery injection of cyanide, markedly alters the respiratory response to the vertebral injection (fig. 1). Its latent period is decreased, and it assumes more of the abrupt and intense nature of the carotid reflex as elicited by direct common carotid injection of cyanide or sulfide. The partial carotid occlusion bringing about such an alteration is usually indicated by a reflex rise in blood pressure resulting from the decreased carotid pressure (Siciliano, 1900; Hering, 1927; Heymans, 1929, 1930; Heymans and Bouckaert, 1930b; Heymans, Bouckaert and Dautrebande, 1931c) as shown at the arrow in the figure.



Figs. 1-7

Direct occlusion of the carotids, as with clamps, during a vertebral injection, decreases the minimum vertebral dose required for a respiratory response frequently to or below the very small order of magnitude of dose required for a carotid reflex effect by direct injection into the common carotid. The latent period may decrease to the order of that of a direct carotid reflex effect. The response to superminimal dosage resembles the direct carotid reflex, except that it is frequently more prolonged. It is commonly even more intense. Such experiments are illustrated in figures 2; 3, A, B, C, D, E; 4, A, B, D, E; 5; 6.

The most obvious immediate interpretation of this alteration in the respiratory response to vertebral injections of cyanide or sulfide by reduction of blood pressure in the carotid system is that the reduced pressure at the carotid reflex zone, together with the reflex rise in general arterial pressure, furnishes a good pressure gradient from the vertebral artery out into the carotid body and sinus, where an ordinary carotid reflex results. The perplexing observation that in many cases the latent period was as short, and the effect more intense, than the reflex effect elicited by direct injection of an equal dose into the common carotid, is conceivably compatible with the circulatory interpretation when one considers that the carotid body, rather than the walls of the sinus proper, appears to be the area of *chemical* sensitivity (Dautrebande, 1931) and is supplied by small branches from the occipital and the carotid bifurcation. Thus, it is possible that there *may* be no appreciable difference in time required for a dose injected into the vertebral artery to enter the carotid body by way of the occipital artery or its branches, from that required for a dose injected directly into the carotid to enter the carotid body by way of the occipital, or the very small branches directly from the bifurcation. As concerns the fact that the altered vertebral response may be even more intense and prolonged than the direct carotid response, it is important to consider that but a fraction of a dose injected directly into a common carotid passes into the small vessels leading to the carotid body, even when the external carotid is occluded. The major part of the dose quickly circulates past the origin of these small branches in a fluid segment probably limited largely to the axial stream. Concerning other circulatory factors, such as would result in a more sustained action of the chemical at the carotid body in the case of the vertebral response, nothing definite can be said.

Vascular isolation of the sinus so complete as to prevent leakage of blood into it when emptied flat usually did not eliminate the abrupt, altered vertebral response associated with obstructed carotid flow. However, it is certainly conceivable that small vessels may have left the arterial branches distal to the ligatures, and supplied the carotid body from the vertebral side. As a matter of fact, the blood supply of that body has been found distressingly variable, and involving many very fine vessels.

A few dissections have disclosed small arteries running through the carotid body region from the occipital artery and sending branches into muscles richly supplied by the vertebral artery.

Occlusion of the occipital artery, while the common carotid was occluded, has been observed to retard the "altered" vertebral response, presumably by curtailing the volume and velocity of flow from the vertebral, the only outlet for blood from the vertebral side, during the occlusion, being into occipital branches.

The shift in the nature of the vertebral response, then, appears to be the result of stimulation of the carotid reflex zone. In spite of such probability that the abrupt vertebral response during reduced carotid pressure is simply a result of collateral circulation, there was still the interesting alternative that the chemical accomplishes the response by central augmentation of predominantly excitatory impulses from the less turgid or non-pulsating sinus, until a series of injections of both cyanide (Winder, Winder and Gesell, 1933) and sulfide (Winder and Winder, 1933) directly into the fourth ventricle was carried out, with the common carotids alternately occluded and patent. No consistent difference in response to such apparently definitely central applications of the chemicals could be observed with the carotid pressure normal or with it reduced. Certainly, by occlusion of the carotids there was no shifting of the nature of the response toward such an abrupt and intense one as we are considering in the vertebral response with carotid pressure reduced. In this connection it should be mentioned that by filling the isolated sinus (but not body) to varying degrees of turgidity, it was not possible to record any consistent alterations in the abrupt vertebral response obtained under such conditions of obstructed carotid flow.

Further, if the marked alteration in vertebral response by carotid occlusion were entirely due to central augmentation of normally rather weak excitatory carotid sinus impulses resulting from the reduced pressure, one should expect that nearly simultaneous injections of *a*, a dose into the vertebral artery such as gives an intense abrupt response with the carotids occluded, (fig. 3, B and C; 4, D and E), though an ordinary intracranial response with the carotids unobstructed (fig. 3, A; 4, B; 7, A and E), and *b*, one of a dose directly into the common carotid that by itself results in a similar response (fig. 3, E; 4, A; 7, B, C and F), would cause a great synergic effect. Such is not the case (fig. 3, F; 4, C; 7, D and G).

These studies reveal that if cyanide or sulfide augments centrally the efficiency of carotid sinus respiratory impulses, the augmentation is of such magnitude that it would have to be shown by a series of experiments statistically treated.

The vertebral-carotid anastomoses which appear to be so definitely emphasized, are of teleological interest. Though the normal pressure

reflexes of the carotid region are altered by carotid obstruction and taken over largely by the cardio-aortic zone (Hering, 1927), the important humoral chemical stimuli arising there, notably the anoxemia stimulus to breathing (Heymans and Bouckaert, 1930c; Heymans, Bouckaert and Dautrebande, 1930, 1931e; Schmidt, 1932), are not lost.

CONCLUSIONS

1. With reservations and precautions, preliminary information may be gained concerning the intracranial action of a chemical or drug by injections into the vertebral artery without carotid denervation.

However, even inadvertent partial occlusion of the common carotid, or other obstruction to its normal flow, throws into play a free vertebral-carotid anastomotic circulation by virtue of which the chemically sensitive carotid body reflex region is in many cases as freely reached by the chemical as though it were injected directly into the normally patent common carotid. Mere occlusion of the common carotids does not eliminate and in many cases probably does not even diminish the rôle that the carotid reflex zone *may* play in the response to a chemical injected into the general circulation.

2. If cyanide or sulfide has a central augmentative influence on carotid sinus respiratory impulses, it is of such magnitude as to require a series of statistically treated quantitative experiments for its study.

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BLOOD CONCENTRATION UNDER THE INFLUENCES OF AMYTAL AND URETHANE

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It is well known that the concentration of the blood with respect to various solid constituents is highly modifiable. Experiments were in progress in which concentrations were being measured (Adolph, Gerbasi and Lepore, 1933) in blood drawn from dogs that were anesthetized by two different anesthetics, sodium amytal and ethyl urethane. It became apparent that the concentrations of the whole blood withdrawn during anesthesia were markedly different in the two sets of dogs, being low under amytal and high under urethane.

Further experiments were now undertaken to measure the development of the changes in concentrations in circulating blood, as shown in single individuals before and during anesthesia. In seeking the source of these differences, spleens were excised and their sizes measured in dogs under each anesthetic. The concentrations of the blood contained in them were also measured. The amounts of possible retention or liberation of concentrated corpuscles in the spleen could thence be compared with the differences found in the circulating blood. Changes in the plasma concentrations also prevailed; these were obviously not due directly to splenic discharge. Observations made during anesthesia of dogs that were previously splenectomized indicated how much of the changes in the blood were due to transudation of fluid and to escape of plasma protein from circulation.

METHODS. Sufficient anesthetic was given to produce deep anesthesia. For sodium amytal (Eli Lilly & Co.) the usual dose was 0.05 gram per kilogram of body weight; a 10 per cent solution was either given by stomach tube or injected intraperitoneally. For ethyl urethane (Merck) the dose was 2 grams per kilogram, a 20 per cent solution being either given by stomach tube or injected intraperitoneally. When the gastric administration produced vomiting, additional urethane was given intraperitoneally.

All the blood analyses reported were performed upon arterial samples. Some of the dogs were operated upon, as soon as anesthesia was deep enough, by cannulating the trachea, exposing and clamping off a brachial artery from which blood samples were to be obtained, connecting a carotid

artery to a manometer, and connecting a jugular vein to a manometer. Samples of blood taken from unanesthetized dogs were drawn from a femoral artery by direct puncture, while the dog lay without struggling on a warmed table; in a few instances arterial blood pressures were read at the same time by connecting the puncture needle to a mercury manometer.

The samples of blood were analysed by six different methods which related the concentrations to various constituents. The methods of analysis,

TABLE I
Concentrations of arterial blood taken from dogs that had been given anesthetic by peritoneum

In each case the analyses given were done on the first sample of blood drawn.

ANESTHETIC	DOG NUMBER	TIME AFTER ADMINIS- TERING	PLASMA PROTEIN	PLASMA SPECIFIC GRAVITY	PW	BT	BLOOD SPECIFIC GRAVITY	BW
		minutes	per cent					
Amytal.....	Fe	83	7.56			1.00	1.0599	3.57
	Ff	85	6.81	1.0321		1.11	1.0574	3.81
	Fg	101	8.05			1.36	1.0570	4.11
	Fl	82	6.92	1.0269	13.1	1.26	1.0570	4.06
	Fp	86	5.94	1.0246	13.7	1.57	1.0518	4.55
Mean.....		87	7.06	1.0279	13.4	1.26	1.0566	4.02
Urethane....	Fj	95	6.34	1.0285		0.52	1.0676	2.88
	Fk	105	7.46	1.0267	11.4	0.49	1.0713	2.83
	Fi	141	7.09	1.0301		0.50	1.0709	2.82
	Fm	91	7.42	1.0299	11.3	0.57	1.0741	2.92
	Fn	151	7.93	1.0291	10.2	0.58	1.0702	2.96
Mean.....		117	7.25	1.0289	11.0	0.53	1.0708	2.98
None.....	Fl		7.20	1.0271	11.8	0.96	1.0613	3.54
	Fp		6.59	1.0255	12.0	1.14	1.0575	3.99
	Fo		7.23	1.0266	10.6	1.06	1.0593	3.79
	Fm		6.73	1.0284	11.3	0.90	1.0632	3.47
	Fn		7.23	1.0275	10.8	1.03	1.0600	3.66
Mean.....			7.00	1.0270	11.3	1.02	1.0603	3.69

The ratios *PW* and *BW* = weight of water evaporated ÷ weight of dry residue, in plasma and in whole blood respectively. The ratio *BT* = (100 - per cent RBC volume) ÷ per cent RBC volume.

with their mean errors and mean physiological deviations, are outlined in the preceding paper. The six kinds of analyses were: refractive index of plasma, specific gravity of plasma, dry residue of plasma, hematocrit, specific gravity of whole blood, and dry residue of whole blood. The analytical results are to be compared by computing the units of liquid found per unit of some constituent that could for the purpose be thought

of as relatively constant in absolute amount. This ratio increases with dilution and decreases with concentration in every case.

The circulating blood. The concentrations found in arterial blood samples taken from dogs under various conditions of anesthesia are shown

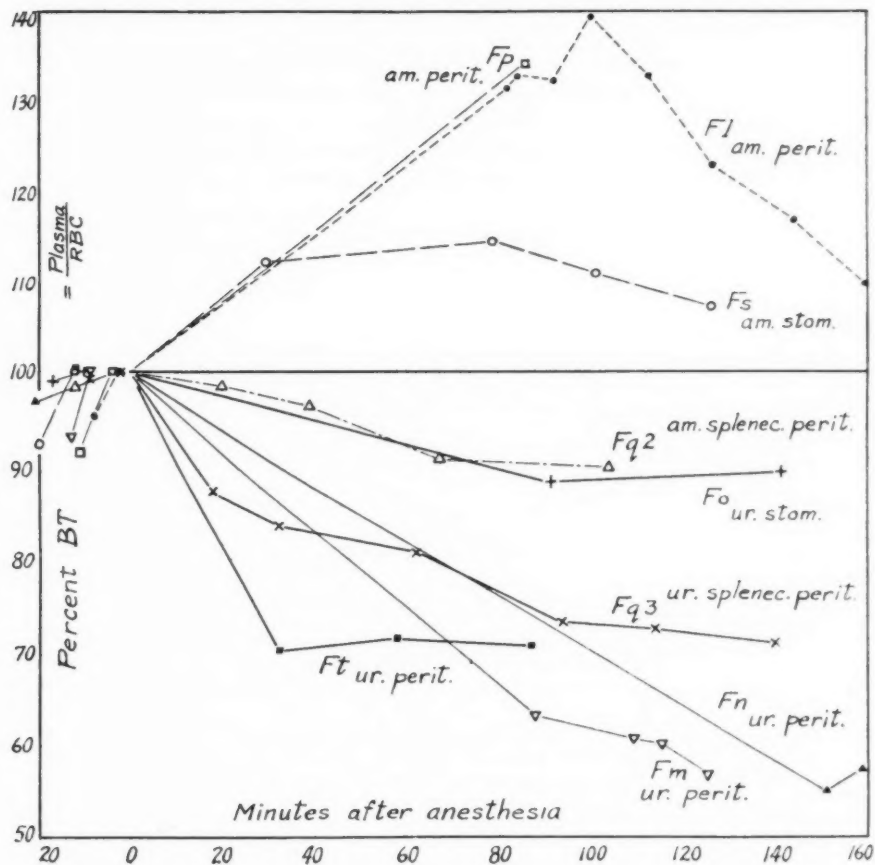


Fig. 1. Progressive changes in blood dilution of dogs as measured by hematocrit. Increased dilution raises the ordinates (*BT*). With the number of the experiment is indicated the anesthetic (amytal or urethane), the route of administration, and absence of the spleen.

in table 1. It is evident that after administration of the two anesthetics by intraperitoneal injection the concentrations of whole blood differ markedly. Taking the blood of unanesthetized dogs as standard, amytal

increased the ratio of plasma to red corpuscles, while urethane decreased this ratio.

With respect to the plasma the data of table 1 exhibit no significant differences, except when blood was sampled from the same individuals both before and after anesthesia.

It was further found that animals receiving either amytal or urethane by stomach had blood concentrations on the borders of the normal range. But those that received urethane both by stomach and by peritoneum showed extreme concentration of the blood.

In a number of dogs the changes of blood concentration were followed in the same individuals before and during anesthesia. With respect to

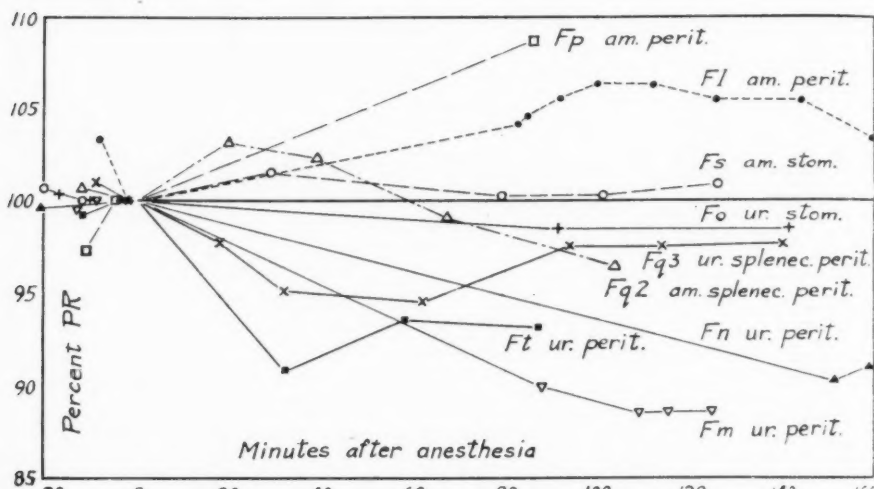


Fig. 2. Progressive changes in blood dilution as measured by refractive index of plasma. Increased dilution raises the ordinates, the ratio *PR* being calculated as $(1 - \text{plasma protein } \%) \div \text{plasma protein per cent.}$ The experiments are the same as those represented in figure 1.

the corpuscles as ascertained by hematocrit measurements, the progressive changes are shown in figure 1. The limits of normal physiological variation are shown by the separation of the values found in the first two analyses. Thereafter anesthetic was administered in the manner indicated for each experiment. It is evident that the change of concentration required 1 to 1½ hours for its development. Significant recovery from the change was shown within 3 hours only in the amytal experiments.

The same relative dilutions following anesthesia were shown by the other methods of whole blood analysis, viz., dry residue and specific gravity.

The results were qualitatively the same for plasma constituents, as indicated in figure 2. This is highly significant, since dilution or concentration of the plasma with respect to its own solid constituents indicates true transudation of fluid. Not only the refractive index, but also the specific gravity and the dry residue of the plasma, showed these marked changes.

Circulatory factors. It is well known that the heart rate increases after amytal has been administered to dogs. In our observation the rate increased equally after urethane, but much more promptly. In both instances the pulse rates were about doubled.

The arterial pressure is known to fall somewhat in dogs anesthetized by amytal (Page and Coryllos, 1926; Stavsky, 1931). It falls more with urethane. The effects of amytal are often classified as paralysis of the

TABLE 2
Weights of spleens and of blood draining from them after excision

ANESTHETIC	DOG NUMBER	BODY WEIGHT	TOTAL SPLEEN WEIGHT	DRAINED BLOOD WEIGHT	DRAINED SPLEEN WEIGHT	DRAINED SPLEEN AS PER CENT OF BODY WEIGHT	DRAINED BLOOD AS PER CENT OF TOTAL SPLEEN WEIGHT	TOTAL SPLEEN WEIGHT AS PER CENT OF BODY WEIGHT	REMARKS
		<i>kgm.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>				
Amytal	Fe	15.4	181					1.17	
	Fe	18.4	261	111	150	0.81	43	1.42	
	Ff	26.3	228	103	125	0.47	45	0.87	
	Fg	9.7	147	56	91	0.94	38	1.51	After transfusion
Urethane	Fh	10.6	40	7	33	0.31	18	0.38	By stomach
	Fj	14.4	38	3	35	0.24	8	0.26	By peritoneum
	Fk	18.5	75	5	70	0.38	7	0.40	By peritoneum
	Fm	16.9	57	6	51	0.30	11	0.31	By both routes

parasympathetic system, but the pressure response does not indicate this. It is more plausible to regard the increase in heart rate as an attempt to compensate for the fall of arterial pressure.

Venous pressure was measured after anesthesia had been induced in a number of dogs. No clear differences were found between the two groups of dogs under the influence of the two anesthetics. The venous pressure as well as the arterial pressure remained constant during the period lasting from 1½ to 3½ hours after administration of amytal by intraperitoneal injection.

The spleen and its blood. The spleen was excised from the anesthetized dog by placing hemostats across the entire mesentery by which the organ was attached, and cutting between the organ and the hemostats. The spleen and all the blood draining from it were caught in a vessel that was

then covered and weighed. After time had elapsed sufficient for the blood to drain from the spleen, the spleen was lifted from the vessel, and the blood that had drained from it was weighed.

The results of these weighings of the fresh excised organ are indicated in table 2. In every instance the spleen was larger in amyralized dogs than in urethanized dogs. The difference is seen to be largely due to blood that drains out of the excised organ, though evidently less blood drains out of the amyralized dog's excised spleen than can be discharged *in vivo* by the urethanized dog. The spleen of the unanesthetized dog evidently contains amounts of blood intermediate to those found under amyral and those found under urethane.

In some instances the blood that drained from the spleen was analysed in the same manner as the circulating blood. In two cases the blood was so rich in corpuscles and hence so poor in plasma that no clotting of it occurred. In other instances blood was taken from the spleen by direct puncture of the organ. In two dogs, four of the samples thus obtained showed significantly larger volumes of red corpuscles per unit of whole blood compared to blood that was circulating at the time; but the remainder showed no differences. Evidently little of the blood in these samples had been obtained from the splenic pulp. In all samples the plasma had the same concentration of solids as the circulating plasma; this was true also of the plasma in blood that drained from the excised spleen, providing it was rigorously guarded from evaporation.

The changes in the circulating blood that would be expected to occur when the spleen discharged can be calculated from these analyses of splenic blood and the data of table 2. The amyralized spleen appears to contain 8 cc. of dischargable blood per kilogram of body weight, this being approximately the difference in mean weight between it and the urethanized spleen. The circulating plasma volume in the dog is about 50 cc. per kilogram (Carrier, Lee, and Whipple, 1922); only about 1 cc. of every 8 cc. stored by the spleen is plasma, and hence when mixed in the circulation increases the plasma volume only 2 per cent. It does not change the plasma concentration directly, and the large changes actually found (fig. 2) point to dilution or concentration of the blood by transudation of tissue fluid.

The circulating corpuscular volume is about 40 cc. per kilogram in the normal dog (Carrier *et al.*, 1922), somewhat less in the amyralized one, perhaps 36 cc., and hence in the urethanized one about 43 cc. When from 3 to 7 cc. of corpuscles per kilogram are put into the circulation by the spleen's discharge in response to urethane, the concentration of whole blood increases markedly. The increases in each of the types of concentration measured in whole blood, when calculated by this procedure, simulate but do not nearly equal the increases of concentrations actually measured

in the bloods of anesthetized dogs. Thus, the calculated increase under amytal in the hematocrit ratio (BT) is 7 to 15 per cent; the actual increase is 34 to 39 per cent (fig. 1). Under urethane the expected decrease is 5 to 11 per cent, the actual decrease is 40 to 45 per cent. Obviously the spleen has only a small part of the capacity necessary to change the blood's concentration by the amount measured.

A splenectomized dog (Fq) was prepared under ether anesthesia. Two and five weeks later the animal was given amytal and urethane respectively. Under amytal the blood did not become diluted with respect to corpuscles (fig. 1) but did for a brief period with respect to plasma proteins (fig. 2). Under urethane the blood concentrated almost as markedly as in the dogs with spleen intact.

It is evident that the spleen is responsible for a considerable part of the disappearance of corpuscles from the circulating blood under amytal anesthesia. But it is responsible for only a small part of the increased concentration of corpuscles in the circulating blood under urethane anesthesia. It is indirectly responsible for a small part of the dilution of the plasma under amytal, possibly because it diminishes the circulating blood volume and thereby the mean capillary blood pressure. It is not responsible, even indirectly, for much of the increase of plasma concentration under urethane.

Extirpation of the spleen was not followed by significant changes in blood concentration or in blood pressures of already anesthetized dogs.

Transudation and changes of capillary permeability. While the spleen is not the only reservoir for blood, it is the only one in which the corpuscles are highly concentrated compared to the plasma. Reservoirs for plasma without corpuscles do not exist in significant amounts within the blood system with the possible exception of the non-axial portions of all minute vessels; but extravascular spaces are well understood to interchange their contents readily and rapidly with the circulating blood.

It is evident in our experiments that fluid was coming into the blood after administration of amytal, diluting the plasma proteins and thereby the corpuscles as well. If the corpuscles in circulation were constant in absolute total volume, the amount of fluid gained or lost by the plasma might be calculated. This condition is very nearly met in the splenectomized dog, and it is largely the case for the dog with spleen intact while under urethane anesthesia, since it has been concluded above that the discharge of the spleen can account for only a small portion of the increase in hematocrit values. The changes in the ratio BT , of plasma to red corpuscles, as shown in figure 1 and in table 3, are then direct values for the amounts of transudation.

Further, the concentration of protein contained in the transudate can be approximately calculated, again on the assumption that the total absolute

volume of circulating red corpuscles is constant. The method of computation is indicated by the formula:

$$\text{Transudate protein \%} = \frac{(BT_1 \times \text{initial pl. protein \%}) - (BT_2 \times \text{later pl. protein \%})}{BT_1 - BT_2}$$

The results are shown, for particular points in each of the experiments, in table 3. They serve to bring out the fact that the transuded fluid is rich in proteins. The computation has been carried out to include the amytal experiments, where the fluid that transudes is entering the blood; but it must be borne in mind that under amytal the spleen causes an enormous change in absolute volume of circulating red cells and that in actual fact

TABLE 3
Concentrations of protein in transuded fluids, calculated from hematocrit and plasma refractometer measurements

ANESTHETIC	EXPERIMENT NUMBER	TIME AFTER ANESTHETIC	INITIAL PLASMA PROTEIN	LATER PLASMA PROTEIN	BT ₁	BT ₁ - BT ₂	TRANSUDATE PROTEIN	REMARKS
		minutes	per cent	per cent			per cent	
Amytal . . .	Fp	86	6.59	5.94	1.573	-0.485	4.23	By peritoneum
	Fl	100	7.20	6.77	1.360	-0.400	5.75	By peritoneum
	Fs	126	7.26	7.33	1.705	-0.117	(8.30)	By stomach
	Fq2	104	6.61	6.83	1.288	+0.141	4.61	Splenectomized
Urethane . . .	Fq3	32	6.81	7.13	1.259	+0.243	5.14	Splenectomized
	Fq3	94	6.81	6.98	1.100	+0.402	6.32	Splenectomized
	Fq3	140	6.81	6.96	1.070	+0.432	6.41	Splenectomized
	Fo	141	7.24	7.33	0.944	+0.120	6.50	By stomach
	Fn	151	7.23	7.93	0.582	+0.463	6.12	By both routes
	Fm	115	6.73	7.53	0.541	+0.358	5.50	By both routes
	Ft	32	6.73	7.36	0.586	+0.247	5.26	By peritoneum
	Ft	87	6.73	7.20	0.589	+0.244	5.62	By peritoneum

whatever fluid enters the circulation probably contains little or no protein. Attempt could be made to correct the values of table 3 for the splenic factor, but the data are clearly too approximate to permit of further manipulation; however, the experiments on the splenectomized dog stand free of such limitation.

The immediate factor leading to transudation is likely to be the decrease (under amytal) or increase (under urethane) of the mean capillary blood pressure. Further factors under amytal may be the reduction of circulating blood volume by the inflation of the spleen, the general lowering of arterial blood pressure, and possible changes in the venous circulation. The greater dilution of the blood when the amytal is injected intraperitoneally may point to the viscera as the site of most of this entrance of fluid

into the blood. The actual amount of fluid put into the cavity with the amytal is negligible in this connection.

The responses to urethane are of quite a different order from those to amytal. The corpuscles instead of occupying 50 per cent of the volume of a sample of arterial blood come to occupy 60 per cent or more of it. The splenic deflation furnishes at most enough corpuscles for one-fourth of this change. The remainder is almost certainly due to transudation of fluid from the plasma to the tissue spaces. This is a deduction not lightly resorted to, but, as in the case of histamine, the changes of blood concentration can be accounted for at present in no other way.

Evidently, too, certain of the capillary blood vessels have become more permeable to plasma proteins. As in the case of histamine, this increase of permeability may be due to a specific effect of the urethane, or it may be an effect of the lack of oxygen that results from the stasis.

It is well known that urethane dilates blood capillaries under some conditions and simultaneously concentrates the blood by loss of fluid from the plasma. In the frog it was found by Landis (1927) that local applications of urethane raised the capillary blood pressure by partial venous obstruction, thus forcing the fluid out of the vessels. It is likely that such a rise of pressure is present in the dog when the urethane is administered intraperitoneally, and that large quantities of water escape from the plasma at this site. Apparently the concentration of urethane present in the general circulation is hardly sufficient to produce the same response in all regions of the body. It may be suggested, too, that the lowering of general arterial blood pressure in the dog by urethane is partly due to the dilatation of capillary blood vessels and rise of the pressure within them in the visceral region.

Other tissues. A number of analyses of dry residue were made upon samples of muscle, liver, and skin obtained under the two anesthetics. In each of these two groups, amytalized and urethanized, the variation of dry residue from dog to dog is so great that comparisons can hardly be made. The average values under each anesthetic were not significantly different, so that it is impossible to say whether the change in blood concentration is accompanied by changes of concentration in other tissues. If the absolute amounts of fluid lost from the circulating blood were distributed with approximate equality among all the tissues of the body, no one tissue would show a measurable change in water content.

COMMENT. Effects of amytal upon blood concentration were looked for in dogs by other investigators. Drabkin and Edwards (1924) stated that "in several control experiments we have shown that this anesthetic has little effect upon blood concentration." The dilution of whole blood and of corpuscles was noted by Bourne, Bruger and Dreyer (1930). They suggested that the effect might be due to the storage of corpuscles in the

spleen, but made no observations of the spleen. They noted, however, that another barbiturate narcotic, luminal, increased the splenic volume. The increase of size of the spleen was measured by Cook and Rose (1930) in decerebrate cats that were being anesthetized with amytal. The surface area of the flattened spleen doubled during the few minutes required for anesthetizing the animals.

The increase in concentration of hemoglobin and red cells in response to urethane was found in rabbits by Boycott and Price Jones (1922). At that time the rôle of the spleen in regulating this concentration was not recognized. Ether and nitrous oxide in anesthetic doses produced the same effects, so that they believed they were observing some general effect of anesthetics upon transudation.

In our experiments urethane and amytal changed the composition relatively little when administered by stomach, but extremely when administered by peritoneum. Since the concentration of anesthetic administered is the same, and approximately the rates of absorption are alike, the difference suggests that the injection has a local action within the peritoneal cavity. This action might also bear upon the spleen, either upon its coat or upon the blood vessels or nerves supplying it, or upon the adrenal glands, whose more rapid discharge of adrenalin is well known to cause the spleen to deflate.

There are indications that many anesthetics, particularly ether, chloroform, and nitrous oxide, simulate urethane in its effect upon blood concentration. Obviously any anesthetic that modifies capillary permeability to plasma proteins is dangerous in physiological studies of water distribution as well as in surgical procedures where circulating blood volume is to be conserved. The factor of anesthesia has been sparingly recognized in the studies of the action of histamine (Dale and Laidlaw, 1919; McDowall, 1923); and observations of our own show that relatively little plasma is lost from the blood when histamine is administered to dogs during *amytal* anesthesia.

Our studies indicate that the anesthesia of choice for studies of water redistribution in the dog is amytal given by stomach. For studies of splenic response, amytal is desirable, since the spleen is not in a *condition* to respond while the dog is under urethane, ether or chloroform.

The blood that is stored in the spleen is well understood to be very rich in corpuscles. We have found that the *plasma* in the spleen does not differ in concentration of proteins from the plasma in circulation. This fact seems unfavorable to the suggestion (Barcroft and Florey, 1928) that concentration of the blood occurs in part through formation of lymph from the plasma that escapes from the interstices among the red blood corpuscles. In addition, lymphatic vessels are generally believed to be absent from the splenic pulp.

The treatment of dogs by amytal constitutes an experiment of importance for the study of transudation, in which surgical interference is avoided. Administration of the anesthetic lowers the mean capillary blood pressure so that water enters the plasma. This effect is the physiological equivalent of hemorrhage (Adolph, Gerbasi, and Lepore, 1933).

Treatment by urethane yields an experiment that stands in marked contrast. The mean capillary blood pressure is raised so that water leaves the plasma. This effect reproduces the changes of blood concentration found to follow the plethora caused by transfusion of blood. The effects of transfusion will be described in a subsequent paper.

SUMMARY

The circulating blood of dogs is greatly diluted after sodium amytal anesthesia and greatly concentrated after ethyl urethane anesthesia. This is due only in part to the inflation and deflation of the spleen respectively. When the anesthetic is administered by stomach tube instead of intraperitoneally the change in the blood is small.

The plasma is diluted or concentrated in the same direction with the corpuscular constituents, though to smaller degrees. This change of plasma concentration is in large part due to interchanges with tissue spaces. In the case of urethane considerable protein escapes from the plasma with the water.

We are indebted to Mr. A. A. Parry for assistance in several of the experiments.

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VISUALIZATION OF THE WALLS OF THE GALL BLADDER

AN EXPERIMENTAL STUDY

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The experimental technic presented here permits radiologic study of the gall bladder without substituting its normal contents with substances which are opaque to the roentgen rays, but differ from bile from a physical and chemical standpoint. Such an attempt was made by Winkelstein (6) who, by suturing four small silver disks to the wall of the gall bladder, made the position of these walls visible roentgenologically. However, this technic did not enable him to detect any changes in the size of the gall bladder.

In a previous experimental investigation on cats, one of us (Gianturco) attempted to visualize the walls of the stomach roentgenologically by means of metallic markings, and found that the silk sutures used to hold the markings in place often caused adhesions and induced immobility of the viscus. Satisfactory results were finally obtained by slipping small chilled lead shots into pockets made in the serosal coat of the organ.

The same technic was applied to the gall bladder of cats, taking care to place the shots in a plane perpendicular to the direction of the roentgen rays. The animals were trained to lie quietly for hours. After the operative wound had healed, observations on the gall bladder were made. After a twenty-four hour fast, the cats were fed 30 cc. of various kinds of food mixed with barium sulfate. This enabled us to follow the gastric digestion while the shots visualized the behavior of the gall bladder. Fluoroscopic observations and plates were taken every ten minutes for the first two hours, and then at hourly intervals until the stomach appeared to be completely empty. By plotting the changes in the distance between two opposite shots in relation to the time intervals, curves were obtained which can be read as those made by a kymograph.

The gall bladder was studied after the injection of water, starch, peptone, Boyden (1) meal (egg and cream) and cotton seed oil. As the curves show, our results agree with those of previous investigators (Boyden, 2; Higgins and Mann, 5; Graham and Cole 3, 4) in that although the size of the organ was slightly diminished after the ingestion of water, starch,

or peptone, the most marked effect was noted by feeding a mixture of egg yolk and cream. Cotton seed oil did not induce marked changes in the size of the gall bladder; after the Boyden meal there was definite diminution in size (figs. 1 and 2).



Fig. 1. Influence of the ingestion of egg and cream (Boyden meal) on the size of the gall bladder. *a*, soon after ingestion; *b*, three hours after ingestion; *c*, six hours after ingestion.

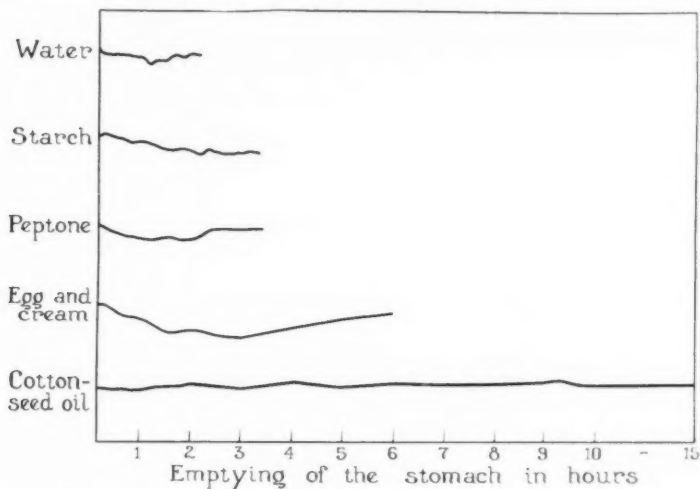


Fig. 2. Correlation of the size of the gall bladder to the gastric digestion of various foods.

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STUDIES ON PROLAN-INDUCED OVULATION IN MIDBRAIN AND MIDBRAIN-HYPOPHYSECTOMIZED RABBITS¹

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Many investigations have shown the significance of the function of the anterior lobe of the hypophysis in the ovulation produced by the injection of prolan. The relationship of the nervous system to this mechanism needs to be considered. It has been shown beyond peradventure that ovulation induced by the injection of pregnancy urine occurs in the complete absence of the visceral afferent and efferent vagal, the thoracolumbar and sacral pathways to and from the abdominal and pelvic viscera (Hinsey and Markee, 1932). To the best of our present knowledge, the only innervation received by the anterior lobe of the hypophysis is by way of fibers from the carotid plexus. Greving (1931) states that there is a group of fibers (Tractus supraoptico-hypophyseus) which passes from the nucleus supraopticus to the posterior lobe of the hypophysis. However, it is conceivable that the hypothalamus may exert an indirect influence upon the anterior lobe.

Hill and Parkes (1931) have shown that prolan produced ovulation in hypophysectomized decerebrate (level not given) rabbits when the injection was made 10 minutes after the removal of the hypophysis. They made no attempt to remove all of the hypothalamus. In the first group of our experiments, we have removed all of the diencephalon and cerebral hemispheres in such a manner as to leave the hypophysis intact. These served as a control for a second series in which the hypophysis was entirely removed in addition to the extirpation of the hypothalamus.

METHOD. These experiments were performed upon New Zealand white rabbits of various sizes (2 to 5 kgm.). The rabbits were isolated in each case for at least three weeks before the experiments were begun. Ovulation was induced by the intravenous injection of 10 cc. of untreated pregnancy urine (approximately six times the minimal ovulating dose). The rabbits were anesthetized with urethane. The calvarium was exposed and removed over the cerebral hemispheres back to the tentorium of the

¹ This investigation was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University and a grant from the Sex Division of the National Research Council.

cerebellum. The superior longitudinal sinus was ligated in two places, sectioned between the ligatures, and the dura was then cut and retracted. The section, extending from the rostral border of the superior colliculi to the point of exit of the III nerve, was made with a blunt spatula and care was taken to avoid, as much as possible, injury to the arteries on the ventral surface of the brain stem in the region. The diencephalon and cerebral hemispheres were then removed and hemorrhage controlled with cotton pledgets. In the midbrain preparations with the hypophysis in place, the cranial cavity was loosely packed with dry cotton. In the midbrain preparations where the hypophysis was removed, the diaphragm of the sella turcica was dissected in such a manner as to render the dorsal surface of the hypophysis completely visible. The hypophysis was completely removed by suction, and the sella was then completely packed with bone wax, after a thorough search had been made to be sure that no hypophyseal remnants had been missed. The animals were left tied on the animal board in a prone position with the caudal portion of the body slightly elevated to prevent seepage of body fluids into the subpontine region. The animals were kept in a warm room. Rectal temperatures were recorded at 30 minute intervals and the body temperatures were maintained between 37 and 39° throughout the progress of the experiments. The urine was injected intravenously at varying intervals following the operative procedures as will be described later in relation to the results obtained. From time to time, sterile saline was injected subcutaneously to maintain fluid balance. In some of the animals, it was necessary to repeat the urethane injections to maintain anesthesia, due to the fact that in its absence these animals exhibited periods of violent activity consisting primarily of running movements.

At the completion of the experiment, a careful autopsy was made. The ovaries were removed and fixed in Bouin's fluid, later to be serially sectioned and stained with hematoxylin and eosin. In each case the macroscopic observations as to the presence or absence of rupture points was controlled by microscopic verification. The number of rupture points reported in the tables represents the number found in a study of a complete series of sections of the entire ovary. The brain stem was removed and fixed in formalin, following the recording of the exact level of the section. A careful search of the sella turcica was made, and only those animals are reported in which the hypophysis had been completely removed. Furthermore it should be recalled that the sella was carefully examined immediately after removal and that the sella was tightly packed with bone wax throughout the subsequent portion of the experiment. In the midbrain non-hypophysectomized series, only those animals are reported in which a blood-supply to the hypophysis was established by means of an intra-arterial injection of India ink.

OBSERVATIONS. *Midbrain non-hypophysectomized animals.* Table 1 illustrates the results obtained when prolan was injected into midbrain animals in which the hypophysis was left in situ. It is evident that ovulation occurred in every instance in both the large and small animals regardless of the interval between the removal of the hypothalamus and the injection of pregnancy urine. It is very evident that the hypothalamus

TABLE 1
Midbrain rabbits

NUM- BER	WEIGHT	INTERVAL	DURA- TION	RIGHT OVARY	LEFT OVARY	REMARKS
	<i>kgm.</i>		<i>hours</i>			
4	3 2	10 min.	14½	5 R.P.*	4 R.P.	Died
1	3 5	30 min.	20	5 R.P.	1 R.P.	Killed
3	4 0	30 min.	25	6 R.P.	3 R.P.	Killed (ovulation time 12-13 hours)
5	4 0	30 min.	13½	6 R.P.	2 R.P.	Died (ovulation time under 12 hours)
2	4 0	1 hr., 35 min.	35	6 R.P.	3 R.P.	Killed (corpora lutea being formed)
13	4 2	5 hrs.	14½	4 R.P.	3 R.P.	Died
14	4 2	5½ hrs.	22	3 R.P.	4 R.P.	Killed
10	2 8	5½ hrs.	11½	4 R.P.	2 large foll.	Died
12	5 0	5½ hrs.	15½	3 R.P.	4 R.P.	Killed. 12 (2 weeks old) corpora lutea
11	4 4	6½ hrs.	18½	6 R.P.	4 R.P.	Killed
23	2 0	5 min.	16½	4 R.P.	4 R.P.	Killed
22	2 1	7 min.	18½	4 R.P.		Killed
26	2 0	7 min.	22½		3 R.P.	Killed (Animal pregnant. Cor- pora lutea pres- ent)
24	2 1	10 min	14½	Very small	2 R.P.	Died
25	2 2	10 min.	19½	2 R.P.	0	Killed
27	2 0	10 min.	16	1 R.P.	2 R.P.	Killed

* Rupture points.

and the visceral centers contained therein play no essential rôle in the production of ovulation by the injection of prolan in the rabbit.

Midbrain hypophysectomized rabbits. Table 2 contains the observations recorded when the hypophysis was removed in addition to the diencephalon and cerebral hemispheres. Ovulation occurred in all the large animals when the interval between the removal of the hypophysis and the urine injection was not more than two hours. At longer intervals, the incidence of ovulation in the larger animals decreases markedly. In the small

animals of this same species weighing from 2 to 2.3 kgm. (table 3), ovulation was never seen to occur following the removal of the hypophysis, in spite of the fact that short intervals were utilized. Reference to table 1

TABLE 2
Large midbrain hypophysectomized rabbits

NUMBER	WEIGHT	INTERVAL	DURATION	RIGHT OVARY	LEFT OVARY	REMARKS
	kgm.		hours			
11	3.0	5 min.	29	3 R.P.	4 R.P.	Killed
9	2.6	10 min.	19½	2 R.P.	3 R.P.	Killed
6	2.8	15 min.	13	4 R.P.	5 R.P.	Died
12	2.9	25 min.	30	3 R.P.	2 R.P.	Killed
23	2.6	35 min.	18	2 R.P.	1 R.P.	Died
13	3.4	38 min.	13	1 R.P.	2 H.F.*	Died
2	3.1	45 min.	13½	0	3 R.P.	Died
24	3.6	2 hrs.	21½	2 R.P.	1 R.P.	Killed
14	3.4	3¼ hrs.	20½		1 M.R.P.†	Killed
21	2.7	3¼ hrs.	12½	2 R.P.	2 H.F.	Died
25	3.2	3½ hrs.	21¼	1 H.F.	2 H.F.	Died
17	3.0	3½ hrs.	14½	1 R.P.	4 R.P.	Died
16	2.9	3½ hrs.	16½			Died
29	2.6	4 hrs.	14½			Died
15	3.0	5 hrs.	12			Died
27	3.4	5½ hrs.	18½	1 H.F.	1 H.F.	Killed
28	4.0	6½ hrs.	15	1 R.P.	1 R.P.	Died
26	3.0	9¼ hrs.	18¼			Died

* Hemorrhagic follicles.

† Mechanical rupture point.

TABLE 3
Small midbrain hypophysectomized rabbits

NUMBER	WEIGHT	INTERVAL	DURATION	RIGHT OVARY	LEFT OVARY	REMARKS
	kgm.	min.				
1	2.1	5	18 hrs.	—	—	Died, very small ovaries
32	2.0	7	21½ hrs.	—	—	Killed
31	2.2	10	18½ hrs.	—	—	Died. Tracheal obstruction
33	2.2	12	18 hrs.	—	—	Killed
10	2.1	20	21½ hrs.	—	—	Died
30	2.3	39	12 hrs. 6 min.	—	—	Died

will show that in a similar series of small midbrain animals with the hypophysis in place, there was ovulation in all cases. Furthermore, control injections in three normal unoperated animals, weighing 2.0, 2.1 and 2.1 kgm. respectively, produced ovulation in every instance. This cer-

tainly would show that animals of this size were capable of ovulation as long as the hypophysis was intact, irrespective of the presence or absence of the diencephalon and the cerebral hemispheres.

Microscopic preparations of the ovaries of these hypophysectomized animals showed that the rupture points were apparently normal, although there was considerable variation in the size of the follicles which ruptured. It is an exceedingly interesting fact that in every ovary of these hypophysectomized animals where ovulation did not occur, there were follicles which contained ova in which the first polar body had been formed and in some cases the second maturation spindle. Apparently, considerable follicular growth had occurred. Around each rupture point there were areas of dilated blood vessels. Similar areas of vasodilatation were not observed in the unruptured follicles.

COMMENT. These observations give conclusive evidence that the hypothalamic visceral centers are not essential for the production of ovulation by the injection of pregnancy urine. An interesting outgrowth of the original observations has been the relationship between the presence of the hypophysis and its blood supply and the occurrence of ovulation. We have been able to confirm completely the observations of Hill and Parkes (1931) and of White and Leonard (1933) who showed that ovulation occurred when prolان was injected at intervals up to an hour following hypophysectomy. When the interval following removal of the hypophysis and the urine injection was longer than two hours in our experiments, ovulation occurred in only three of ten experiments (after $3\frac{1}{4}$, $3\frac{1}{2}$ and $6\frac{3}{4}$ hours) in spite of the fact that we were injecting at least six times the minimal ovulating dose. This decrease in the incidence of ovulation is given added significance by the fact that control injections after long intervals (5 to $6\frac{1}{2}$ hours) in five large midbrain rabbits with the hypophysis *in situ* showed ovulation with a large number of rupture points. It would seem that this decrease in the occurrence of ovulation with the hypophysis removed is in some way related to the absence of the hormones of the anterior lobe of the hypophysis. White and Leonard (1933) found that when massive doses of prolان were injected 1, 4, 6, and 6 days after hypophysectomy, only slight stimulation of follicular growth occurred. The absence of a positive statement regarding ovulation would lead to the inference that it did not occur.

Firor and Reynolds (1933) report that the injection of prolان induced ovulation in three animals that had failed to ovulate following hypophysectomy performed 35 minutes after coition. In the absence of a statement in their abstract as to the interval between hypophysectomy and the urine injection, it is difficult to compare their observations with those of White and Leonard (1933) and our own. Furthermore, this comparison is not justified since in all three of their animals some of the gonad stimulating

substance may have been liberated in the 35 minutes that elapsed between mating and hypophysectomy. If this were the case, it may explain the presence of ovulation in their series while in our experiments, when the interval was longer than two hours, ovulation was the exception rather than the rule. In relation to these experiments upon the rabbit, it is significant that Reichert et al. (1931) and Kraul (1932) were unable to induce follicular development by the injection of prolan in rats and dogs at long intervals following hypophysectomy.

This decrease in the incidence of ovulation can be explained in at least two ways. In the first place, White and Leonard (1933) have pointed out that the removal of the hypophysis is followed by the degeneration of the ovarian follicles on account of a decreased concentration of hypophyseal hormone in the circulating blood. It is conceivable that these degenerating follicles would no longer react to prolan. However, our histological preparations do not show any apparent degeneration probably because of the fact that they were taken at comparatively shorter intervals. It should also be recalled that considerable development had occurred in these follicles with the formation of the first polar body and, in some instances, the second spindle. It is tempting to suggest that there was insufficient hypophyseal hormone present to complete the process of ovulation. This in turn suggests the second possible explanation, i.e., in these large animals there may be a continued elaboration of hormones from the anterior lobe of the hypophysis that remain in the organism for a comparatively short time after hypophysectomy. The decreased concentration of these hormones may explain the ineffectiveness of prolan for causing ovulation after longer intervals.

Contrary to the findings with the larger animals, it was observed that, when the hypophysis was removed from the smaller animals of the same species, ovulation did not occur even when the injection was made a few minutes after the extirpation. The controls in midbrain small animals with the hypophysis in place ovulated in every instance. This certainly seems to point to the hypophysis as the source of at least some of the activating principle causing ovulation when prolan was injected. It appears that, in these smaller animals, which were just becoming sexually mature, the hypophyseal hormones might be resident in the hypophysis itself and be liberated upon the injection of prolan. When the hypophysis was removed in these animals, the available hypophyseal hormones in the tissues may have been at low ebb and consequently ovulation did not occur, in spite of the fact that considerable follicular growth was induced. This interpretation is in keeping with the observations of Smith and Engle (1927) and of Wolfe and Cleveland (1931), who found about as much gonad-stimulating hormone in the hypophyses of immature as in those of mature animals.

Evans, Meyer and Simpson (1932) believed that prolan was unable to function except in combination with hypophyseal hormone. They attributed the ovulation observed by Hill and Parkes following hypophysectomy as due to a combination of the injected prolan with minimal amounts of hypophyseal hormone still circulating in the blood of these animals. This seems to be a logical explanation for our observations of ovulation in the hypophysectomized large midbrain animals with shorter intervals, its decrease in incidence with longer intervals and its absence in hypophysectomized small midbrain animals with short intervals. Nevertheless, we do not wish to doubt the possibility of the correctness of the other explanation which has been advanced nor to deny that there may still be other ones. However, we feel that the evidence indicates that the presence of hypophyseal hormone in the tissues of the rabbit is essential for the production of ovulation by the injection of prolan.

SUMMARY

When prolan was injected into midbrain rabbits with hypophyses in place, ovulation occurred regularly in both large and small animals, regardless of whether short or long intervals (10 minutes to 6½ hours) elapsed between the operation and injection. Its injection into midbrain hypophysectomized large rabbits produced ovulation in all cases when the interval between hypophysectomy and the injection was less than two hours. When this interval was longer than two hours, the incidence of ovulation decreased in these animals (3 out of 10). In small midbrain hypophysectomized animals, it was impossible to cause ovulation by prolan injection even at short intervals. The possibility of the necessity of a minimal amount of hypophyseal hormone in body fluids for the occurrence of prolan-induced ovulation is discussed.

It is a pleasure to acknowledge the coöperation we have received from Dr. Edward Liston and Dr. Russel Lee and his associates of Palo Alto, California.

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THE QUANTITATIVE SEPARATION OF PROGESTIN FROM OESTRIN IN EXTRACTS OF THE CORPUS LUTEUM

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In a series of publications emanating from this laboratory we have described the preparation of corpus luteum extracts which, among numerous other properties, have the ability to produce in the castrated adult rabbit progestational proliferation of the endometrium, and which, when injected in adequate dosage into rabbits castrated 18 hours after mating, will maintain pregnancy to full term. The hormone responsible for these changes we have named progestin. This hormone differs from oestrin physiologically in that the alterations it induces in the uterus cannot be brought about by oestrin, and chemically in that it is readily destroyed by saponification with alkali.² However, it has been impossible up to the present time to demonstrate that progestin will produce its characteristic effects in the absence of oestrin because all extracts of the corpus luteum made in this laboratory, and presumably those made by other workers, contain oestrin in addition to progestin. Such contamination with oestrin is to be expected since this substance can be prepared by exactly the same method as that used for making progestin and since it is present in considerable amount in the ovary. Whether that present in the extracts is elaborated by the corpus luteum or whether it represents a leakage from the adjoining follicles is a disputed point, but the fact remains that corpus luteum extracts contain oestrin.

In this paper we propose to describe in detail a procedure for the separation of the oestrin from the progestin in such extracts without the destruction of either hormone. Such a separation is of more than academic interest because relatively oestrin-free progestin is necessary in many physiological problems related to reproduction and because, without a means of separation, progestin cannot be sought in the urine and placenta of preg-

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² The various papers dealing with the details of preparation, means of assay, yields of hormone and physiological properties of progestin are listed in the bibliography as follows: Corner, 1928; Corner and Allen, 1929; Allen and Corner, 1929; Goldstein and Tatelbaum, 1929; Allen, 1930a, 1930b; Allen and Corner, 1930; Allen, 1932a, 1932b; Reynolds and Allen, 1932; Meyer and Allen, 1933b.

nant women since extracts made from these sources contain so much oestrin that an accurate assay of progesterin, were there any present, would be well nigh impossible. (See W. M. Allen, 1932a, for data and literature bearing on the inhibition of progesterin by oestrin.)

The method we have found satisfactory for this separation is based upon certain data obtained in separating cholesterol from progesterin. It was found that, after extracting a 70 per cent methyl alcohol solution five times with one-third its volume of petroleum ether (B.p. 30–55°), approximately 75 per cent of the progesterin remained in the alcohol whereas practically all of the cholesterol and about 25 per cent of the progesterin were found in the petroleum ether (W. M. Allen, 1932 b, table 3). By subjecting the results of this distribution to mathematical analysis³ we find progesterin to be approximately five times as soluble in 70 per cent alcohol as in petroleum ether under the conditions of the experiment. Prior to this Ralls, Jordan and Doisy (1926) had shown that oestrin was much more soluble in 70 per cent alcohol than in petroleum ether, their results (table 4 b, preparations 8, 10, 12) indicating that an average of at least 96.6 per cent of the oestrin remained in the alcohol after this solution had been extracted six times with one-fourth its volume of petroleum ether. Subjection of these results to mathematical analysis similar to that used in the determination of the solubility of progesterin in 70 per cent alcohol shows oestrin to be at least 40 times as soluble in 70 per cent alcohol as in petroleum ether. These wide

³ The formula we have employed for this analysis is

$$X_n = A \left(\frac{kb}{1 + kb} \right)^n$$

in which

X_n = amount of progesterin in 70 per cent alcohol after n extractions with petroleum ether.

A = amount of progesterin present before extraction with petroleum ether.

$k = \frac{C_a}{C_p}$; C_a = concentration of progesterin in 70% alcohol.

C_p = concentration of progesterin in petroleum ether.

$b = \frac{V_a}{V_p}$; V_a = volume of alcohol.

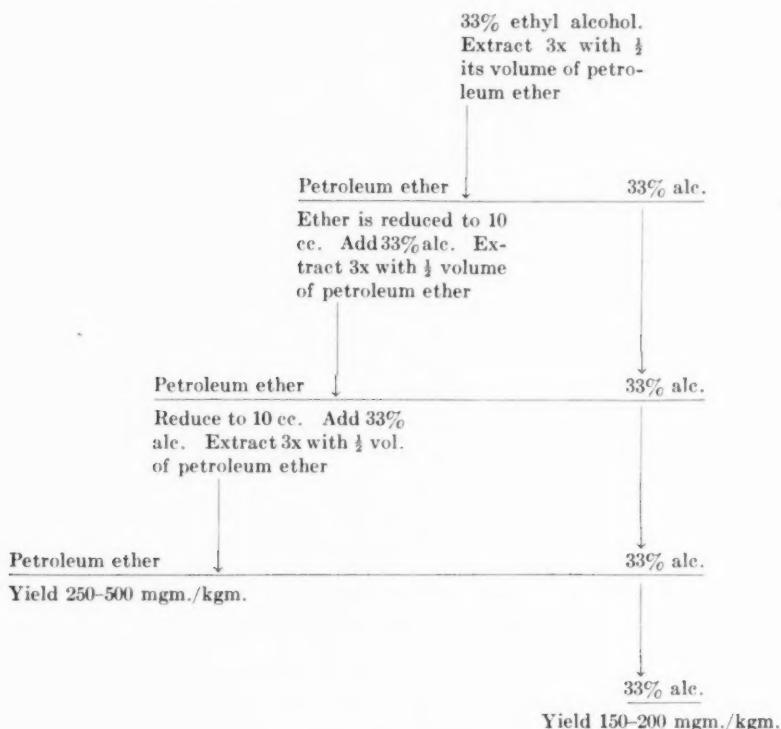
V_p = volume of petroleum ether.

This formula is the standard distribution formula ordinarily used for the study of the distribution of a substance between two immiscible solvents. The relative solubility of the hormone in the two solvents is represented by k , i. e., the concentration in alcohol divided by the concentration in petroleum ether.

differences in the distribution ratios of oestrin and progestin of course make a separation theoretically possible but at the same time rather impractical because of the relatively high solubility of both substances in 70 per cent alcohol as compared to their slight solubility in petroleum ether. However, they (Thayer, Jordan and Doisy, 1928) have subsequently shown that 92 per cent of the oestrin remains in 50 per cent alcohol after extracting five times with one-fourth its volume of petroleum ether. This fact seems to make a separation practically as well as theoretically possible since we have found that progestin can be extracted from 50 per cent alcohol with petroleum ether if large volumes are used. We chose, therefore, to extract 33 per cent alcohol with petroleum ether, thereby hoping to get a high percentage of progestin in the petroleum ether and at the same time a high percentage of the oestrin in the alcohol. How completely this hope was realized is shown in tables 1 and 2. It is seen at a glance that we obtained 100 per cent of the progestion and only 5 per cent of the oestrin in the petroleum ether fraction and 95 per cent of the oestrin and practically no progestin in the 33 per cent alcohol fraction.

The detailed procedure for an extract of 1500 grams of dissected corpus luteum tissue is as follows: fraction 1 (W. M. Allen, 1932b, p. 595), the ethyl ether fraction containing both oestrin and progestin, instead of being washed with aqueous NaHCO_3 as indicated in that scheme, is reduced to dryness by boiling off the ether and the residue is dissolved in 80 cc. of absolute ethyl alcohol. Two volumes of distilled water (160 cc.) are added thus reducing the alcohol content to 33 per cent. When the water is added the solution becomes opalescent. The dilute alcohol solution is then extracted three times with one-half its volume (120 cc.) of petroleum ether (purified over conc. H_2SO_4 and redistilled, Bp. 30-55°C). This single procedure secures an excellent separation of the two hormones but we repeat the procedure twice more. The petroleum ether extracts are combined, reduced to 10 cc. by boiling, 80 cc. of absolute alcohol and 160 cc. of water are added and the resulting 33 per cent alcohol solution after being extracted three more times with 120 cc. portions of petroleum ether is then added to the first 33 per cent alcohol. Finally, to secure as good a separation as is practically possible we repeat the process a third time. From the theoretical standpoint it would be better to extract more times with smaller volumes of petroleum ether but practically it is easier and quite satisfactory to use the volumes we have indicated. These steps are perhaps clarified by the scheme shown on page 58.

The progestin-containing petroleum ether is boiled off and the residue is dissolved in 25 cc. of absolute or 95 per cent ethyl alcohol. There is considerable white, amorphous, insoluble material which may be removed by centrifugation if desired but for physiological studies this is unnecessary since it contains neither oestrin nor progestin and it is non-toxic. Ordi-



narily the yield is 250 to 500 mgm. per kilo and 1 rabbit unit of progestin is equal to 10 to 20 mgm. of solids. The oestrin-containing 33 per cent alcohol is distilled in vacuo to about 10 cc. and the aqueous remainder, which in our experience is always acid to litmus, is extracted three to four times with 50 cc. portions of ethyl ether. The ether fraction is then distilled to dryness and the residue dissolved in 10 cc. of 95 per cent alcohol. The yield is usually 150 to 200 mgm. per kilo and 1 rat unit of oestrin is equal to 1.9 to 2.3 mgm. of solids.

The results of the progestin distribution are summarized in table 1. It should be said that only preparations 95, 96 and 97 were carried out exactly as described above. The others, however, were carried out by such closely related methods that they can be included in the table. For example, 94 was extracted from 33 per cent alcoholic solution with one-third its volume of petroleum ether rather than one-half and 93 was extracted from 33 per cent alcohol five times rather than three times. In general it may be said that the distributions in the earlier separations were more

TABLE 1
Distribution of progestin between petroleum ether and 33 per cent ethyl alcohol

BEFORE SEPARATION					PETROLEUM ETHER FRACTION					33% ETHYL ALCOHOL FRACTION				
Preparation number	Equiva- lent of fresh tissue injected	Solids	Proliferation*	Preparation number	Equiva- lent of fresh tissue	Solids	Proliferation	Progestin, per cent original	Preparation number	Equiva- lent of fresh tissue injected	Solids	Prolifera- tion	Progestin, per cent of original	
88	34	33	+++	88-A	44	12	+++	100	88-13	112	100	0	<14	
92-A	33	345	+++	92-A-a	33	9	+++	100	92-A-b	132	0	<13	<13	
	22	230	++		22	6	++							
	22	230	+		33		+++							
A-8-b	34	141	+++	A-8-b-1	34	118	+++	100	A-8-b-2	395	0	<4	<4	
	23	94	+		22	78	++							
93-E	112	68	+++	93-H	109	46	+++	100	93-1	1687	320	0	<2	
	75	45	+++		75	32	+++							
	37	23	++		37	16	++							
94-E	96	33	+++	94-H	96	12	+++	100	94-1	1648	260	+	2.5	
	42	14	0		69	9.8	+++							
95-E	61	25.4	+++	95-H	46	10.6	+++	100	95-1	652	115	0	<3.5	
	45	18.6	++		23	5.3	++							
96-E	60	32	+++	96-H	40	22.6	+++	100	96-1	842	121	0	<2.5	
	42	26.2	++		30	17	+++							
97-E	43	27.8	+++	97-H	43	20	+++	100	97-1	1000	164	0	<2.2	
					23	11	+							

* For illustrations showing the degree of proliferation indicated by these + signs, see W. M. Allen, 1930a

complicated than those of the later ones but the recovery of the progestin was equally satisfactory in all cases for without exception 100 per cent was recovered in the petroleum ether fraction. The apparent recovery of more progestin after separation than was supposedly present before separation (table 1, prep. nos. 94, 95, 96) is probably due to the fact that the oestrin present before separation inhibited to some extent the development of endometrical proliferation by the progestin. These data by no means prove this but it is a plausible explanation for these unexpected results since it has been adequately demonstrated that sufficient dosage of oestrin will completely suppress the proliferating ability of progestin. (Tausk, de Fremery and Luchs, 1931; Leonard, Hisaw and Fevold, 1932; W. M. Allen, 1932a.)

TABLE 2
Distribution of oestrin between petroleum ether and 33 per cent ethyl alcohol

Preparation number	PETROLEUM ETHER FRACTION					33% ALCOHOL FRACTION				
	Equivalent of fresh tissue per mouse	Degree of* mucification produced	Equivalent of oestrin	Rat units of oestrin per kgm.	Per cent of total oestrin	Preparation number	Equivalent of fresh tissue per rat	Number of rats injected	Per cent of rats in oestrus	Per cent of total oestrin
	<i>grams</i>		<i>rat units</i>				<i>grams</i>			
93-H	253	+++	0.46	3.6	5.7	93-I	16.8	20	55	94.3
	126	++								
94-H-a	137	++; strat.	0.66	4.8	6.2	94-I	13.7	20	55	93.8
95-H-a	131	++	0.46	3.5	4.0	95-I	12.2	16	50	96.0
96-H-a	135	++	0.46	3.4	4.1	96-I	12.6	16	56	95.9
97-H	133	++	0.46	3.4	4.8	97-I	14.5	12	42	95.2

* For illustrations showing the degree of mucification indicated by these + signs, see Meyer and Allen, 1933a.

The distribution of the oestrin is recorded in table 2. The thirty-three per cent alcohol fraction contained approximately 95 per cent of the oestrin and the petroleum ether fraction about 5 per cent. It may be argued that these should be expressed in terms of that present before separation rather than as percentages of the total amount present in the two fractions; as a matter of fact, we have determined in one extract the oestrin present before separation as well as that after separation and we have found that the same equivalent of fresh tissue from each fraction brought 50 per cent of the rats into heat in each case. This result would of course indicate a 100 per cent recovery of the oestrin in the 33 per cent alcohol fraction. We feel, however, that there is some oestrin present in the petroleum ether fraction since it

will produce mucification of the vagina when a sufficiently large dose is given (Meyer and Allen, 1932; 1933a).

The results of this method of separating oestrin from progestin can perhaps be more directly stated in another way. To obtain a figure of < 2 per cent for the progestin in 93-I it was necessary to give 1687 grams equivalent of fresh tissue and, since this produced no proliferation at all, it was assumed that 3374 grams did not contain more than 1 Rb.U. This dose, therefore, is 90 times the amount from the petroleum ether fraction necessary to produce a +++ proliferation. Similarly, a figure of 5.7 per cent for the oestrin present in 93-H means that 126 grams equivalent of fresh tissue produced a ++ mucification, this in turn being estimated as 0.46 rat unit, whereas 16.8 grams of the 33 per cent alcohol fraction contained 1 rat unit. In this case, therefore, 16.5 times as much oestrin was present in the 33 per cent fraction as in the petroleum ether fraction.

The progestin was assayed by the method previously described (Corner and Allen, 1929). Sexually mature female rabbits were mated, castrated 18 hours later, and then injected once daily for the next five days with the desired amount of solids dissolved in 1 cc. of Mazola oil (commercial maize oil), 0.2 cc. being injected daily. Autopsy was performed on the day after the last injection and the uteri were all submitted to histological study, the degree of proliferation obtained being expressed as 0, +, ++, +++, +++++ (W. M. Allen, 1930a).

The oestrin in the 33 per cent alcohol fractions was assayed upon adult castrated rats. Since the method of injection has been shown to have a marked effect upon the results obtained we shall give in detail our method of assay. From 12 to 20 rats were used for each assay. They were injected three times at 12 hour intervals with the desired amount of hormone dissolved in 10 per cent alcohol. (Our preparations were stored in 95 per cent alcohol and dilutions were made from this stock solution the day injections were started.) A rat unit was considered as the amount necessary to induce a fully cornified smear in 50 per cent, ± 10 per cent, within 48 hours after the last injection. A single series of three injections was given once weekly and no assay was considered as final unless at least 50 per cent of those injected had been in oestrus the preceding week. This method of assay has proven very satisfactory and it has given a moderately sharp endpoint. For example, preparation 94-I gave 70 per cent positive with 16.7 grams equivalent of fresh tissue, 55 per cent positive with 13.7 grams, and 10 per cent positive with 8.3 grams.

The oestrin present in the petroleum ether fractions (i.e., the progestin fraction) was assayed by an entirely different method, it being impossible to assay the very minute quantities of oestrin present by the standard rat method without the use of huge amounts of tissue. An example suffices—to assay the oestrin present in 95-H-a (285 gr = 1 r.u.) by the standard

rat method using 20 rats would require about 5,700 grams of fresh tissue equivalent assuming that a 50 per cent result were obtained with the first injection. Such a means of assaying the oestrin being obviously out of the question, we decided to inject a single castrated mouse with the desired dose uniformly injected for the first 8 days immediately following an artificially induced oestrus. When an oestrin preparation is injected in this manner (Meyer and W. M. Allen, 1933 a, table 2) a variety of results may be obtained depending upon the dose of oestrin given. If a large dose is given (2-3 r.u. in 8 days) the animals come into oestrus during the course of the injections and the vagina at autopsy on the 9th day is cornified. If only 1 r.u. is given they do not come into heat, as far as can be determined by daily vaginal smears, but the vaginas at autopsy are either cornified or stratified. Now, when the total dose is reduced to from 0.5 to 0.6 r.u. a +++ mucification results, and when reduced still further to from 0.3 to 0.5 r.u. a ++ mucification is produced. This method, although probably less accurate than the standard rat method, gives us a series of significant results each of which may be interpreted in terms of rat units and consequently a means of assaying small amounts of oestrin in a single animal. We considered no assays by this method as final unless a + or ++ mucification was produced since this fraction (Meyer and Allen, 1933b) contains an inhibiting principle which prevents any oestrin present from producing cornification, at least with any doses which we have given (up to 250 grams, i.e., about 20 times the equivalent of a rat unit of the 33 per cent alcohol fraction). It may be contested that the use of mucification as a means of identifying the oestrin is fallacious but if we had not used such a means of assay our figures of separation would be even better than those given, since we have found absolutely no oestrin in any of our petroleum ether fractions as far as could be determined by the induction of vaginal cornification. It cannot be categorically stated, however, that they would not produce heat if extremely large doses were given.

DISCUSSION. The important question of whether this relatively simple procedure will secure separation of oestrin from progesterone if the preliminary extraction and purification are carried out by methods other than those we have employed cannot be answered from any data which we now find available. Our own experience has been that if separation is attempted with cruder fractions than those we have used the results are very unsatisfactory. There is no difficulty in obtaining a good yield of progesterone in the petroleum ether but there is great difficulty in getting much of the oestrin to remain in the 33 per cent alcohol. This is probably due to the fact that both oestrin and progesterone are fat soluble and that the presence of much lipid in a fraction affects their distribution. Similarly, we are unable to state whether extraction with acid alcohol rather than neutral alcohol would in any way affect the separation. However, we have found

that it does not materially affect the solubility of progestin in organic solvents, and there seems no reason to believe that it would alter the solubility of oestrin, so we might suspect that the procedure we have given would be satisfactory provided most of the fatty substances had been previously removed.

It will be noted from table 1 that the yields of hormone in the last five preparations are only from one-half to two-thirds of those in the first three. This is not due to any change in or fault with the method but rather to a different source of the raw material. The better yields are in agreement with those previously reported from this laboratory (W. M. Allen, 1930 a) and were obtained from tissue secured from the local abattoir on the day of killing and preserved in alcohol on the afternoon of the same day, whereas the poorer yields were from tissue collected and preserved in exactly the same manner but received from a more distant packing company. We have no adequate explanation for these differences in yield since we have obtained practically identical yields of extracted solids from both sources.

SUMMARY

A method for the quantitative separation of progestin from oestrin in extracts of the corpus luteum without the destruction of either hormone is described in detail. The procedure is based upon the fact that progestin is more soluble in petroleum ether than in 33 per cent ethyl alcohol and oestrin more soluble in 33 per cent alcohol than in petroleum ether. Since the results obtained are quantitative in nature the method makes possible for the first time an accurate study of the various effects of each of these hormones practically free from the other in their relation to many of the physiological problems of reproduction.

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THE RELATION BETWEEN THE POSITION OF EXPERIMENTAL MYOCARDIAL LESIONS IN THE DOG AND THE CHANGES IN THE RS-T SEGMENT OF THE ELECTROCARDIOGRAM

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Experimental evidence pointing to a relation between myocardial lesions and T-wave changes in the electrocardiogram was first given by Eppinger and Rothberger (1909). Two years later Kahn (1911) found negative T-waves following coronary artery ligation. Smith (1918 and 1920) definitely established the relationship between myocardial infarctions and T-wave and RS-T deviations. The relation of these changes to clinical cases was pointed out by Herrick (1919), Pardee (1920), and Willius (1922).

Parkinson and Bedford (1928) classified the RS-T changes following coronary occlusions into the well-known T_1 and T_3 types. In their clinical cases, Barnes and Whitten (1929) found that changes in the electrocardiogram of the T_3 type were associated with lesions of the posterior basal portion of the left ventricle and the adjacent septum while involvements of the anterior apical portion of the same ventricle gave electrocardiograms of the T_1 type. Since infarction of the right ventricle was so rare, conclusions were not reached in regard to this condition.

Barnes and Mann (1932) concluded from their work on ligation of the coronary arteries of the dog that the changes in RS-T following acute infarction of the left ventricle of the dog closely resemble the changes in RS-T following acute infarction of the anterior apical portion of the left ventricle of man; and that changes in RS-T following acute infarction of the right ventricle of the dog resemble the changes following acute infarction of the posterior basal portion of the left ventricle of man. They did not demonstrate any difference in the effect upon the RS-T of infarction of various portions of the same ventricle.

In an attempt to correlate the position of myocardial lesions with the resulting electrocardiographic changes, Crawford, Roberts, Abramson and Cardwell (1932) studied the effect of lesions in various portions of the wall of the two ventricles of the cat. The lesions were produced by cautery. The heart was divided into eight regions, viz., anterior apical, anterior basal, posterior apical and posterior basal of the two ventricles respectively.

The curves obtained were classified according to the T_1 and T_3 types. Lesions in the anterior wall of the left ventricle resulted in curves of the T_1 type, while those in the posterior wall of the same ventricle produced curves of the T_3 type. Lesions of the right ventricle, except those of the anterior basal portion resulted in curves of the T_3 type. Lesions of the anterior basal portion of the right ventricle resulted in only slight changes.

Since the question has never been clearly answered as to whether experimental infarctions of various portions of a given ventricle in dogs produce unlike changes in the RS-T segment, we wish to report on an attempt to correlate the position of definite lesions in the two ventricles with the RS-T changes.

METHODS. Radon seeds which consisted of radium emanation in sealed glass capillary tubes were inserted in various portions of the myocardium with the idea that by this means the area of destruction could be chosen at will. The lesion thus produced could be very accurately located and its position did not depend upon any variability in blood supply. The strength of these seeds varied from 3 m.c. to 10 m.c., the usual strength being about 4 to 6 m.c.

Under ether anesthesia administered by means of an artificial respiration apparatus and a tracheal tube, rib resection was performed and the heart exposed by means of pericardial incision. The radon seed was inserted by means of the usual applicator. In the majority of cases the heart was touched by nothing but the needle, while in the others the visceral pericardium was grasped gently with blunt forceps in order to expose the desired area. In no case was the heart removed from the pericardium. The chest was closed with the lungs expanded to fill the thorax. Electrocardiographic tracings were taken before operation, daily for the first 5 to 10 days after operation and thereafter at irregular intervals.

In presenting the data, only the changes in the level of the RS-T segment from the isoelectric level are used. Barnes and Mann (1932) have pointed out that changes in the direction of the T wave are common in normal dogs and that these changes may occur in control animals in which the operation including pericardial incision is carried out just as in the experimentals except that no infarction is produced. Our experience agrees with that of these workers.

In control experiments 5 dogs were used. In 3 of these, the exposure of the heart was carried out just as in the experimental animals including the puncture of the heart wall with the applicator needle. In the remaining 2, the pericardium was opened but the heart itself was not touched.

RESULTS AND DISCUSSION. In studying the data it at once became apparent that the electrocardiographic changes could be classified according to whether the lesion was on the anterior or the posterior aspect of the left ventricle. It seemed to make little difference whether the lesion

involved the base or apex of a given aspect of the ventricle. Such a classification, however, was found to be more difficult in the case of the right ventricle. The results may be summarized as follows:

1. Lesions on the anterior aspect of left ventricle, 8 dogs. This group also includes cases in which the lesion was located on the antero-lateral aspect of the left ventricle near the base. In lead I, all cases showed an elevation of R-T. In lead II, 4 dogs showed an elevation of R-T, and 4 no change. In lead III, 5 showed a depressed S-T, 2 an elevated R-T, and 1 no change. These results are included in table 1 (a).

TABLE 1

The effect of lesions of various portions of the myocardium upon the RS-T segment of the electrocardiogram (figures represent number of dogs)

DAYS AFTER OPERATION	LEAD I			LEAD II			LEAD III		
	Elevated RS-T	Depressed RS-T	No change	Elevated RS-T	Depressed RS-T	No change	Elevated RS-T	De- pressed RS-T	No change
(a) Lesion on the anterior aspect of the left ventricle, 8 dogs									
1-10	8	0	0	4	0	4	2	5	1
11-20	0	0	5	0	1	4	0	1	4
21-	0	0	3	0	0	3	0	0	3
(b) Lesion on the posterior aspect of the left ventricle, 7 dogs									
1-10	0	0	7	6	0	1	6	0	1
11-20	0	0	4	1	0	3	1	0	3
21-	0	0	2	0	1	1	0	1	1
(c) Lesion on the anterior aspect of the right ventricle, 8 dogs									
1-10	4	0	4	1	3	4	0	5	3
11-20	2	0	5	0	3	4	0	3	4
21-	1	0	3	0	1	3	0	2	2
(d) Lesion on the posterior aspect of the right ventricle, 7 dogs									
1-10	2	2	3	1	1	5	4	2	1
11-20	1	0	4	0	0	5	1	1	3
21-	0	0	1	0	0	1	0	0	1

2. Lesions on the posterior surface of left ventricle, 7 dogs. In lead I, all showed no change. In lead II, 6 showed an elevated R-T, and 1 no change. In lead III, 6 showed an elevated R-T, and 1 no change. Table 1 (b) includes these results.

3. Lesions on the anterior surface of right ventricle, 8 dogs. In lead I, 4 dogs showed an elevated R-T, and 4 no change. In lead II, 1 showed an elevated R-T, 3 a depressed S-T, and 4 no change. In lead III, 5 showed a depressed S-T, and 3 no change. These results are found in table 1 (c).

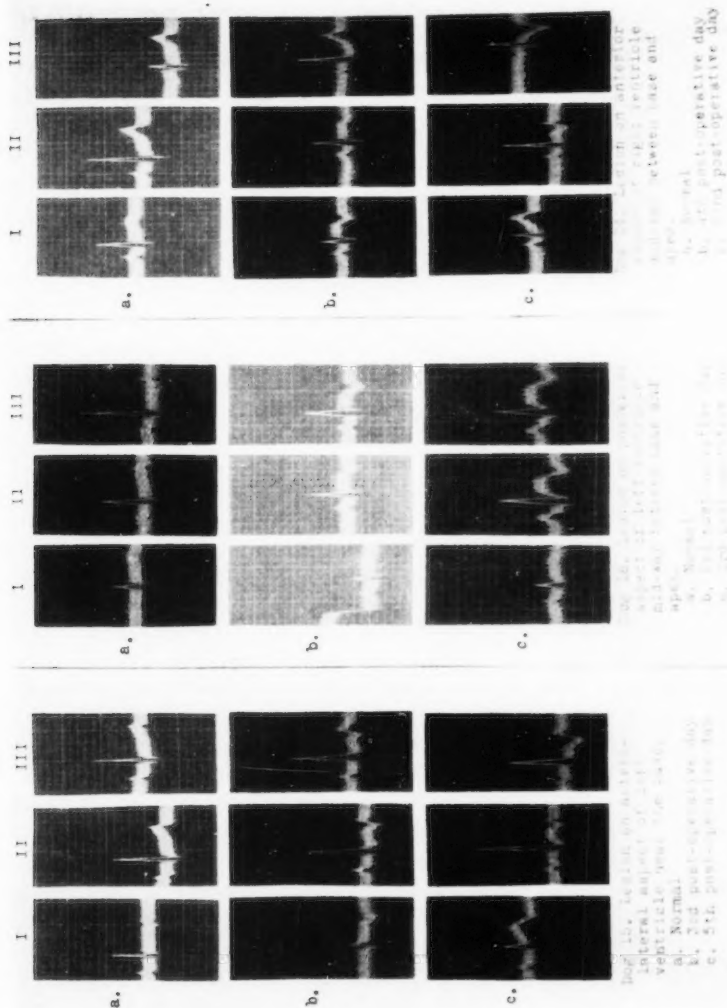


Fig. 1

Fig. 2

Fig. 3

4. Lesions in the posterior aspect of the right ventricle, 7 dogs. In lead I, 2 showed an elevation of R-T, 2 a depressed S-T, and 3 no change. In lead II, 1 showed an elevated R-T, 2 a depressed S-T, and 5 no change. In lead III, 4 showed an elevated R-T, 2 a depressed S-T, and 1 no change. These results are found in table 1 (d).

Illustrations of the results of lesions on the anterior aspect of the left ventricle, the posterior aspect of the left ventricle, and the anterior aspect of the right ventricle may be seen in figures 1, 2, and 3. Since the results obtained from lesions on the posterior surface of the right ventricle were more variable, illustrations have not been included.

Usually the changes in RS-T shown in the above four groups occurred before the fifth post-operative day. In some cases these changes occurred as late as the tenth day. In most cases there was no change at the end of the first 24 hours. Within twelve hours after operation the dogs were able to walk about. A return of the electrocardiogram to normal commonly occurred 5 to 15 days after operation. Neither the extent of the electrocardiographic changes nor the time of return to normal could be correlated with the extent of myocardial damage.

5. Control group, 5 dogs. In one of the two dogs in which the heart was exposed and the myocardium punctured with the applicator needle, a few pericardial adhesions were found at autopsy over the area where the surface of the heart had been punctured. This dog showed no significant changes in the level of the RS-T segment. In two of the five control animals, a slight deviation of the RS-T from the isoelectric level was noted, but in no case were the changes comparable to those observed in the experimental group of animals.

It would seem therefore that the changes observed in the electrocardiograms of the dogs in which myocardial lesions were produced were caused by these lesions and not by other factors incident to the operation. Adhesions were frequently found between the visceral and parietal pericardium, but they seem to have had little or no effect on the results. One of the control animals, for example, showed pericardial adhesions over a small area comparable in extent to that often observed in the experimental ones, and yet there was no significant change in RS-T.

A study of the results indicates that lesions of the left ventricle in our dogs produced changes which might be divided into the T_1 and the T_3 types, and that the T_1 type is associated with lesions of the anterior wall of the left ventricle while the T_3 type is associated with lesions of the posterior wall of the same ventricle. These observations agree with those of Crawford et al. (1932) who followed the electrocardiographic changes following cauterization of various portions of the myocardium in cats, and with the clinical observations of Barnes and Whitten (1929).

The work of Fowler, Rathe and Smith (1933) in which no correlation

between the location of small infarcts and the consequent RT deviations was found, does not agree with our findings. It is entirely possible that the small infarcts induced by them are not comparable to the relatively large areas of necrosis which were produced in our animals. Lesions of the right ventricle yielded less striking results. In the case of lesions of the anterior surface of the right, however, the only variations from the normal which occurred were those of the T_1 type and were found in five of the eight dogs. Lesions of the posterior surface of the right ventricle resulted in no changes which could be correlated with the position of the lesion, unless the fact that 4 of the 7 dogs in this group showed an elevated R-T in lead III, a change of the T_3 type which might be considered significant.

A possible point of interest in connection with the lack of significant RT or ST deviations in the presence of relatively large lesions in some of our dogs is given in the recent paper by Wood and Wolferth (1933). They found that when RT and ST changes were slight or lacking entirely in the three standard leads, rather striking changes might be shown in leads from the anterior and posterior chest wall.

A complete histological study of the hearts obtained at autopsy has not been made, but a few sections have been studied and found to show marked hemorrhage and necrosis. The arteries were found to show a marked resistance to the radon effects. Grossly, the lesions appeared as grayish white necrotic areas measuring from 1 to 3 cm. in diameter. In a number of cases the lesions were found to extend entirely through the heart wall. In most cases, the radon was inserted just beneath the visceral pericardium, and the consequent lesions were found to have not grossly involved the innermost portion of the wall. Perforation through the lesion with consequent hemorrhage was a frequent cause of death even after the electrocardiograms indicated recovery. No evidence of infarcts peripheral to the site of the lesions was found.

SUMMARY AND CONCLUSIONS

The changes in the level of the R-T and S-T segments of the electrocardiogram were studied following the production of myocardial lesions in the dog. The lesions were produced by means of radon implants. An attempt was made to correlate the position of the lesion with the changes in the level of R-T and S-T.

Lesions on the anterior surface of the left ventricle consistently produced changes of the T_1 type.

Lesions on the posterior surface of the left ventricle resulted in changes of the T_3 type in six of a total of seven dogs studied. One showed no change.

Lesions on the anterior surface of the right ventricle resulted in changes

of the T_1 type in five of the eight dogs studied. The remaining three showed no change from the normal.

Lesions on the posterior wall of the right ventricle resulted in changes of the T_3 type in four dogs, the T_1 type in two dogs, and no change in one.

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THE EFFECT OF REWARD ON THE RESPONSE TO PAINFUL EXPERIENCE IN THE CONDITIONED REFLEX

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Everyone acquainted with dogs knows that the various breeds exhibit wide differences in physical form as well as in general behavior. On observation it further appears that the characteristic behavior of each breed fits in a very definite manner the form and appearance of the animal and follows what is known in a general way of the modified behaviors of peculiar types of human beings. In the dog, just as in the human, we find giants which have characteristic gait, movements of orientation and voice. Other dogs are small, vivacious, highly excitable and lean in body form, which is comparable again to similar physical types in man. It is of special interest to biologists and students of behavior to determine whether or not dogs of one breed do have in fact a peculiar behavior that is associated with certain physical forms and internal environment. This can best be accomplished by a study of the inheritance of form among the hybrids resulting from crossing the breeds. The correlation of endocrine activity as to size, activity, and influence on internal environment must also be associated with behavior. A systematic analysis of behavior is now being made on such dogs. For the past few years Dr. C. R. Stockard, director of the Cornell Experimental Anatomy Farm, has been conducting experiments on the inheritance of physical form and endocrine gland types among pure breeds and hybrid dogs (1). This material offers special opportunity for a study of behavior in pure breeds and its possible inheritance in the cross bred dogs, and for the past two years we have been making a comparative analysis of behavior in the same dogs used in the inheritance of form experiments. The dogs employed in the behavior studies have been of the following breeds: dachshunds; British Bulls; German Shepherds; and Basset Hounds, as well as first and second filial generations obtained from crosses of these pure breeds. The results of these general studies will be reported later, the present short communication deals only with an investigation of the conditioned avoiding reaction and the use of a reward as an aid in learning, as followed in detail in only two animals. One of the animals is a hybrid obtained by crossing a pure Great Dane with a St. Bernard, this will be referred to as Dane-Bernard. The other dog is a

cross between the German Shepherd and the Basset-Hound, and will be called for convenience, Basset-Shepherd.

Most experimenters have used the conditioned salivary reflex as a basis for analyzing the cortical mechanisms as well as the general inhibitive and excitable nature of the dog. It has been shown by the conditioned salivary reflex, for example, that a dog discriminates between tones as close as 637.5 and 850 vibrations per second (2). Close discriminations have been developed in the tactile analyzer and visual analyzer by the same method. The procedure that has proven most satisfactory is that of contrast, in which the positive signal is always followed by food and the signal which is to be differentiated from the positive is never followed by food. Before finer differentiations are built up, wide discriminations are first made, and then the two tones or tactile points, as the case may be, are brought closer together until the dog can no longer discriminate between them. When this point is reached the analytical ability of the cortex has been determined. In the conditioned salivary reflex the animal makes an adjustment to food taking, thus it may be characterized as an "approach reaction," it in no wise offers danger to the animal or calls for "avoiding reaction." The cortical mechanism which functions during food taking and pleasant experience is the same that functions during adjustment to painful and dangerous environments, and it is of interest to learn whether it performs in the same manner in the two cases. It has been shown by Cannon (3) that under conditions of danger and pain the organism as a whole is modified, sympathetic changes occur leading to release of glycogen from the liver, constriction of blood vessels and changes in coagulation time of blood, etc., all of which aid the organism in adjusting during a dangerous experience. It is important to know how the cortical mechanism is modified, if it is modified at all during this adjustment. The animal may make closer discriminations than it does in a food taking adjustment, or on the other hand generalized response to signals may be so wide that fine differentiations cannot be built up. It is with the behavior of the dogs in a laboratory environment involving danger and pain that we are here interested.

MATERIAL AND METHOD. An apparatus was devised in the conditioned reflex laboratory at the Experimental Anatomy Farm in which the animal could avoid a painful object by making an adjustment to a signal which preceded and was coincident with the painful experience. This was done in order that the motor reflex could be compared with the salivary reflex. The whole function of the cortex in a situation involving danger is to protect, that is, signal the approach of the dangerous object. We supposed that if the animal could make an avoiding adjustment the cortex would function normally, and neurosis would not develop, even though we were using pain to induce the unconditioned response. The particular reflex used in the experiment was the flexion of the right foreleg following an

electric shock. The painful stimulus was applied by a simple electrode buckled around the leg of the animal consisting of a positive and a negative wire from the secondary coil of an inductorium. A string was also fastened to the leg so that when the foot was lifted a sliding contact switch was opened, cutting off the shock. In this manner the animal avoided the shock by raising the foot. The procedure followed in the experiment was to have the signal precede the shock by three seconds, then coincide with the shock for five or more seconds. The conditioned stimuli used consists of two tones with the Dane-Bernard, and two metronome rates with the Basset-Shepherd. The tones were produced by blown bottles, one with a vibration of about 144, as tuned to the piano, which was used as positive, and the other with a vibration rate of 512. Since we were not interested in fineness of differentiation these served our purpose. The metronome rates used with the Basset-Shepherd were 120 as a positive and 50 as a negative. In producing the metronome vibrations a telegraphic sounder was placed in the animal room and a metronome with mercury dip contacts, which was in the experimenter's room, was used as an interrupter. The laboratory in which the conditioned reflex is being studied is constructed of heavy concrete blocks and contains two rooms $8\frac{1}{2}$ by $7\frac{1}{2}$ feet, which are separated by a partition wall of 6 inch concrete. In the partition between the rooms there is a heavy refrigerator door which does not transmit sound. The animal room is lined with celotex set off two inches from the plastered concrete wall allowing a dead air space for insulation. All communication between the rooms is done either electrically by wires set in a sealed pipe through the partition, or pneumatically by tubes running through the pipe. The only exception to this is the string used to record the flexion of the right foreleg when the painful stimulus is applied. This string was run through a long copper tube 2 mm. in diameter, which was also through the sealed pipe in the partition wall, and then attached to a recording lever on the smoked drum. During the experiments the animals remain on a platform only four inches above the floor and are confined by harness before an apparatus which presents food. The food presenting apparatus consists of a revolving table with six aluminum pans. It is hung on a vertical axis and is rotated by means of a string and weight mechanism around the axis. The table is surrounded by a cover which allows only one pan to be exposed to the dog at a time. A noiseless pneumatic break is used to stop the table and give a pan of food to the dog. Since the laboratory is isolated from other buildings these precautions were sufficient to keep out all disturbances. During the experiments a record is made of a smoked drum of the time of presentation of the signal, breathing changes, leg movement, time of presentation of food and a time record in seconds.

Development of the conditioned response. When a dog is shocked on the

leg it raises the leg and removes it from the painful object. We wished to learn whether the animal could be conditioned to hold the leg up during a signal which preceded for a time and was then coincident with the shock. The first response that appears is the simple conditioned flexion of the leg to the signal but the leg is lowered and raised alternately while the shock and signal are given. This response occurred in the Dane-Bernard after applying 15 signals with shock. In the Basset-Shepherd it appeared after 7 applications of the signal with shock, in the case of the metronome, and after 2 applications of tone 1 with shock, which was developed after the metronome had been used for some time. The conditioned sustained response was more difficult to develop. The Dane-Bernard did not begin to hold the leg up during the sounding of the positive tone, of 144 vibrations per second, until after 125 applications of the tone with shock, and even at this stage the leg was not sustained until some shock was received. After 200 conditioned signals had been given the animal would raise the leg as soon as the signal was applied and sustain it during the continuation of the signal. The Dane-Bernard always placed the leg on the floor again after the conditioned signal was stopped. The Basset-Shepherd, a more timid and cautious animal, and evidently more disturbed by the shock, began to hold the leg up after 25 applications of the signal (metronome 120), but instead of placing the leg on the floor when the signal stopped, this animal would hold it off the floor. The two animals developed the conditioned avoiding reaction, but the Dane-Bernard would allow the leg to be lowered during the intervals between the signals, while the Basset-Shepherd would not completely lower the leg. This indicates individuality of adjustment to the same noxious situation. The Basset-Shepherd did not keep the leg raised enough to cut off the shock, in case it had been given during an interval, but high enough to make for a rapid and easy adjustment when the signal was given. This seemed to be a postural adjustment that was preparatory for the avoiding reaction.

Development of differentiation. The negative tone of 512 vibrations per second was not used with the Dane-Bernard until after the positive tone had been applied 239 times, while rate 50 of the metronome was used with the Basset-Shepherd after the positive rate of 120 had been applied 27 times. At this point both of the animals would give a conditioned reaction every time the signal was applied. It is evident that under the conditions of the experiment, where the significant thing is removal of the leg from the painful object, the problem is quite different from that in the salivary reflex in which the negative signal is not reinforced by food and in which inhibition begins to develop at once. In our experiment, due to generalization, the response to the negative tone and metronome was as strong as that to the positive signals, and due to this the dogs would not make an exception to the negative signals by lowering the leg. It was evident that

in order to prevent the animals from raising the leg to the negative signals we would need to add a positive element, leading to another response involving the lowered leg at the same time the negative signal was given, or we would have to lower the leg mechanically by hand. The last method was unsuccessful, and after trying this we added food as a positive factor to the negative stimulus. In order to take food the animals had to lower the leg and approach the pan. The approach to the pan, when the food was first given with the negative signal was made cautiously by each animal. Both of them lowered and raised the leg a number of times, as if exploring to see whether they would receive the shock before turning to take food. Following this initial response the animals would raise the leg when the negative signal was applied, but would lower the leg when the food was presented during the continuation of the negative signal. The response to the positive signal of course remained the same as that described above. It was evident that the dogs were not differentiating between the tones and metronome rates, but between a tone accompanied by food, and a tone accompanied by shock, as well as a metronome rate accompanied by food and one by shock. The response after the addition of food to the negative did not vary in the Basset-Shepherd after 43 contrasts of the two signals. The Dane-Bernard continued to give the same responses to the two after 97 contrasts. The addition of the positive factor to the negative signals led to a response the animals were unable to make before, but true differentiation between the signals had not developed. In the present stage inhibition to the negative signals did not occur until food was seen by the animal.

Since the addition of food to the negative signals caused the animals to lower the leg, a response they were unable to make before this, we added food to both the positive and negative signals to determine whether the animals would then differentiate between the tones and metronome rates. Under these conditions if the Dane-Bernard lowered the leg to take food during the sounding of tone 2 (512) and did not attempt to lower the leg during tone 1 (144) and try to take food, we may assume that the animal was differentiating between the tones. In the case of the Basset-Shepherd, if the leg is lowered during rate 50 of the metronome and food eaten, and not lowered during rate 120, we may assume that this dog is distinguishing between the metronome rates. The method was successful. Of course, at first, each animal attempted to lower the leg and take food when it was presented with the positive signals (generalization), but since the shock was received each time the leg was lowered, the animal sustained the leg and refused food. In this case the painful stimulus, being dominant, led to the sustained leg response, while food taking was inhibited. The Dane-Bernard tried to lower the leg on the first 14 applications of the positive signal with shock. After this the animal would sustain the leg and refuse

food. The Basset-Shepherd attempted to lower the leg on only three applications of the positive signal plus food. During the applications of these positives plus food, negatives were also applied with food, and in

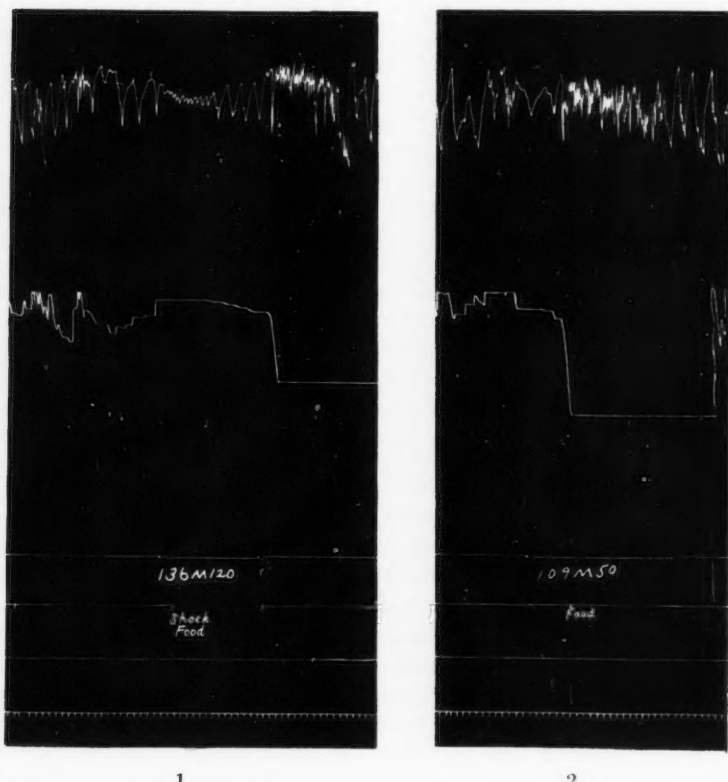


Plate 1. Graphs showing how the Basset-Shepherd differentiates between the two metronome rates. The top line in the graphs indicates breathing changes; the second from the top, leg movement; the third, time of presentation of signal; the fourth, time of presentation of shock and food. The bottom line is a time record in seconds. In graph 1 the animal holds the leg up during M 120, although food is before him. As soon as the M 120 is stopped, the animal lowers the leg and takes food. In graph 2 we observe that the animal lowers the leg when food is presented during the sounding of M 50.

each case the dogs would approach the pan and eat. The response at present in the Dane-Bernard, after giving the positive tone with food 22 times and 12 negatives with food in contrast to them, is in the form of a

raised leg when the positive is given before the shock is applied, and refusal of food. The animal lowers the leg and takes food during tone 2, and in some cases does not raise the leg at all to this signal (see plates 1 and 2). The response in the Basset-Shepherd, which always holds the leg off the floor, is somewhat different. This animal lowers the leg at once when food

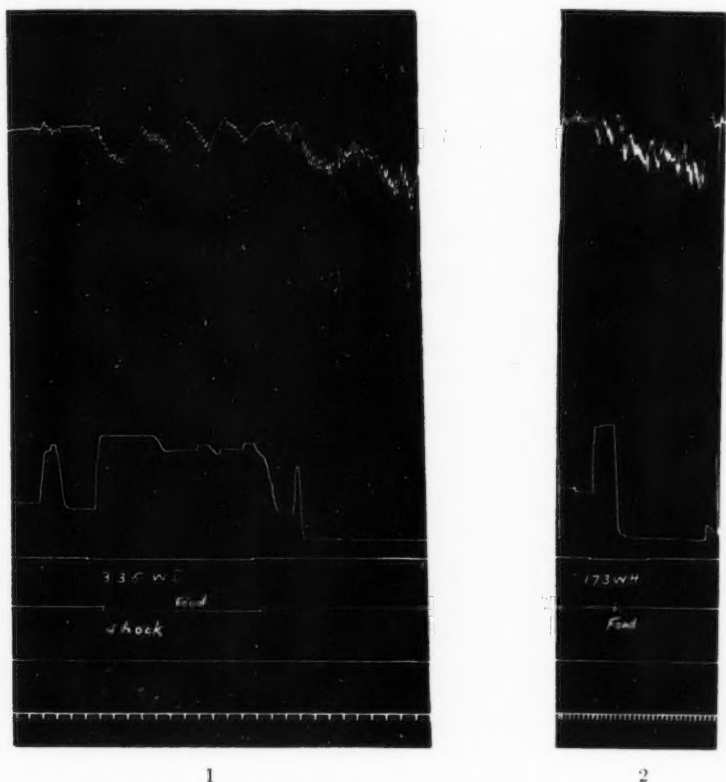


Plate 2. Graphs showing how the Dane-Bernard differentiates between the two tones. In 1, the animal sustains the leg during the sounding of the positive signal and avoids the shock although food is before her. In 2, we observe that she responds by the raised leg when the signal begins, but lowers the leg when food is given, although the signal continues.

is given with rate 50 of the metronome and takes food. When the positive rate is given, however, the animal holds the leg higher and does not look toward the food pan or try to eat. This differentiation occurred and continued after 3 applications of the positive with food in contrast to 5 negative signals plus food.

GENERALIZATION. The general behavior of the animals during the experiments as well as when the negative signals were first applied, suggested that there is greater generalization of the conditioned response in situations involving danger and pain than there is in conditioned food taking adjustments. In order to check this, we presented a number of other signals including a buzzer, bell, and whistles differing from those used in the study. Both animals gave a positive response to each of these signals, and if food was given during the continuation of these signals each animal lowered the leg cautiously "exploratory behavior," and since the shock was not received they would take food. We have trained 18 dogs with the salivary reflex and have not found such wide generalization in any of them. The generalization in the food taking adjustment is only to closely related signals.

DISCUSSION AND CONCLUSION

In the situation dealt with in this experiment the animal makes a conditioned avoiding adjustment. The development of the conditioned response and differentiation between the signals may be outlined briefly as follows:

1. Tone 1 (or M 120) plus shock—Unconditioned flexion of leg, the leg is raised and lowered alternately.
After repetition of this combination:
2. Tone 1 (or M 120)—Conditioned avoiding response, the leg is sustained to avoid the shock.
When tone 2 (or M 50) is added for the first time:
3. Tone 2 (or M 50)—Conditioned avoiding response, generalized response.
Since the leg adjustment here is as intense as that to tone 1 and M 120, the animal does not make an exception, and therefore inhibition does not develop.

When a positive factor, food, is added to the negative signals:

4. Tone 2 (or M 50) plus food—Animal lowers leg cautiously at first, then turns for food, since the shock is not received.

Following 4 we do not have a differentiation between the tones, or metronome rates, because when these signals are applied the animals raise the leg and keep it raised until food is seen before them. They differentiate between a tone accompanied by food and one without food, as well as a metronome rate accompanied by food and one without food. Here we have two inhibitory factors present, namely, inhibition of the sustained leg when food is taken, and inhibition by absence of the painful stimulus. This does not lead to differentiation between the signals, however. When the positive factor is added to both the signals, we have:

5. Tone 1 (or M 120) plus food—First conditioned avoiding response, then the animal sees the food and lowers the leg cautiously. Since in this case the response to the shock, received when the leg is lowered, is dominant, the food taking response is inhibited.

When 5 is contrasted with 4 above, the animals differentiate between the tones and metronome rates by taking food during the continuation of the negative signals, and by refusing food presented with the positive signals. After repetition of this contrast the animals at times do not raise the leg at all to the negative signals.

Since in the above experiments the animals were in a situation of pain and danger we may assume that the strong sympathetic reaction, as indicated by breathing and general behavior, involved suprarenal activity. The shock used was intense enough to make the dog raise the leg vigorously, and at first the whole body was included in the avoiding reaction. After a few repetitions, since the animal could not escape, the reaction was limited to the foreleg. Once the animals had learned to avoid the pain and to respond when the conditioned signal was given, they did not hesitate to enter the laboratory and have not shown signs of nervous disturbance. They appear alert during the experiments and raise the leg to every new sound presented. We have not found such wide generalization in the salivary reflex. For this reason I think we are justified in concluding that in situations involving danger and pain to the organism, generalization of the response to various signals is wider than in situations involving food taking adjustments. We could not develop differentiation between the tones and metronome rates until a positive factor was added which tended to counteract this wide generalization of excitation. This would seem to show that in a noxious situation concentration of excitation to a cortical point does not occur until there is an interplay of two excitatory points leading to antagonistic reactions. When this condition occurs wide generalization breaks down and the animal begins to make finer differentiations.

A further study, that is at present in progress, is to determine whether or not in a situation similar to that in the present experiments the animal makes finer differentiations than in a food taking situation.

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CYCLICAL PHENOMENA PRODUCED IN RATS BY SECTION OF THE PITUITARY STALK AND THEIR POSSIBLE RELATION TO PSEUDO-PREGNANCY

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In 1930 we were able to show that permanent diabetes insipidus could be produced in rats by a stab wound made in the brain stem near the anterior margin of the pituitary gland (Richter, 1930). Since the autopsies in these experiments disclosed impairment of both the pituitary stalk and the brain stem, further experiments were undertaken to demonstrate the effect of injury to either the stalk or brain stem alone. In eighty-five of the three hundred and twenty animals used in these experiments the attempt was made to cut the stalk alone. This proved to be a very difficult task due to the small operative field and the fact that the stalk cannot actually be seen. Five of the animals of this group, in which the operation was successful, that is, in which the stalk was completely severed with little or no damage to the brain-stem, showed a phenomenon which seems to warrant a separate report, quite apart from the diabetes insipidus problem. This phenomenon, consisting in the appearance of regular 9 to 18 day cycles in general activity and food- and water-intake, has furnished the data for this paper.

METHODS. The rats were kept in individual cages of the type used in all our activity studies (Richter, 1927). Each cage contained a revolving drum with a cyclometer, and a living compartment with a food box and water bottle. Running activity in the drum, and food- and water-intake were recorded daily. Vaginal smears were also made each day on most animals, and body weight was recorded weekly. The animals were placed in the cages at about 35 days of age and were allowed a short period for adaptation before operation.

The technique of reaching the pituitary gland through the sphenoid bone has been described in detail in earlier papers (Richter, 1930; Richter and Wislocki, 1930). The bone was approached retro-pharyngeally without entering the buccal or nasal cavities, thus avoiding infection from this source. An opening was made with a dental drill as far forward as possible without entering the sphenoid sinus. Approximately one-third of the

gland was exposed, and the stalk severed with a fine hooked probe which was pushed through the meninges at the anterior lateral margin of the hole and drawn across the midline to the other side.

RESULTS. *A. Cycles in activity.* Activity records of two of the five animals are presented in figure 1, with daily running activity measured in revolutions of the drum indicated on the ordinates and the duration of the experiments in days on the abscissae. The animal whose record is presented in the lower curve was allowed to run for a period of about 40 days before the stalk was cut. During this period the 4 to 5 day cycles associated with ovulation were present though not very regular. Immediately after the stalk was cut, however, the entire character of the activity record

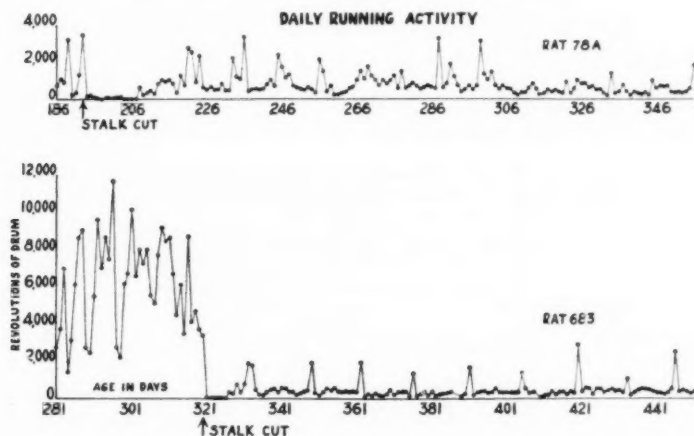


Fig. 1. Daily running record of two females before and after stalk section. Running activity in number of revolutions of the drum is given on the ordinates, the duration of the experiment in days on the abscissae.

changed; there was a marked drop in the level from 6,000 revolutions per day to less than 500, and bursts of activity lasting for a day only occurred very regularly every 14 days. The record at the top, from another animal, shows the cyclical bursts of activity after stalk section, somewhat less regular, and of a slightly longer duration, occurring at short intervals, about every 10 days. The records from the other three animals are similar, although the frequencies are different.

B. Cycles in food and water intake. No less striking are the cyclical changes that appeared post-operatively in the food- and water-intake. It may be seen from the first graph in figure 2 that the changes in the water-intake were very definite and regular, with a range of fluctuation from 33 to 70 cc. per day. That the water-intake cycles are closely paralleled by

food-intake may be seen in the second graph, where food-intake ranges from 8 to 20 grams per day at the extremes of the cycles.

A comparison of these curves with the activity record at the bottom shows that when the animals are most active they eat and drink least. They reach their maximum in food- and water-intake about midway between the activity peaks, and the decrease in food-intake starts usually about 4 days before the burst in activity.

These cyclical manifestations are summarized in table 1, with special reference to regularity and rhythm. It will be observed that in all of the animals, transitory or permanent symptoms of diabetes insipidus were present.

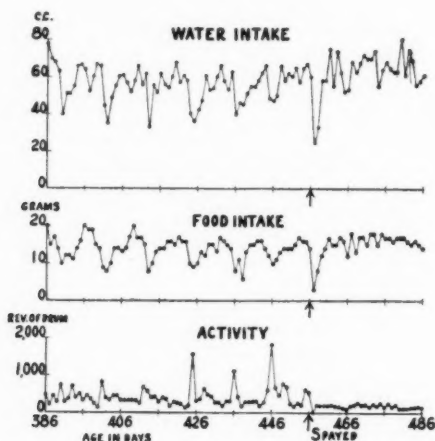


Fig. 2. Food-intake, water-intake and activity records showing regular cyclical changes after stalk section; also showing disappearance of the cycles after ovariectomy.

C. Cycles in vaginal smears. It is worthy of note that only females exhibited these cycles, and that there was a definite relationship between the cyclical changes and the condition of the reproductive tract. This was shown by the fact that cornified smears were present at the peaks of activity, just as they are in the 4 to 5 day cycles, indicating that ovulation must have occurred at that time. Moreover the cycles of food- and water-intake as well as activity disappeared completely after ovariectomy (see fig. 2).

D. Autopsy findings. At autopsy it was found that the pituitary stalk was completely severed in all five animals. The gland itself was injured in varying degrees, but remnants at least of all the components, the anterior, posterior and intermediate lobes, were still present. The pituitary

of the animal with the most regular cycles, of 14 days, showed least injury, being completely intact, so far as could be discovered, except for sectioning of the stalk. Histological studies of the brain stem showed injury in three of the animals, but none were detected in the remaining two.

Autopsy of the other eighty animals in which the attempt was made to cut the stalk disclosed an incomplete section. For the most part the cut was made too far posterior, and to one side, well away from the stalk through the gland proper.

ORIGIN AND SIGNIFICANCE OF THE CYCLES. We are aware that the small number of animals showing the above cycles, as well as the fact that these cycles could not be produced at will, greatly limits a discussion of their origin and significance, and does not justify drawing any general conclusion. In view of the sharp definition and great regularity of the rhythms we do feel, however, that at least a temporary formulation regarding their origin might be made, to serve as a basis for further research.

It is important for this purpose to keep in mind the essential characteristics of the cycles. They occurred with great regularity at frequencies varying from 9 to 18 days with an average of 14 days. Each animal maintained its own rhythm within the given range. And finally, the cycles were observed only in females and were found to be associated with changes in the ovaries, the peaks of activity coinciding with ovulation and disappearing completely after ovariectomy.

It should be noted also that section of the pituitary stalk, which apparently produced the lengthened cycles, may have modified the circulatory connections that pass to and from the gland through the stalk, and that the nervous supply may have been completely interrupted. It is possible that the pituitary gland was freed by section of the stalk from the controlling nervous influences from the sympathetic according to Dandy (1913) and from the midbrain according to Pines (1925). Such a gland could then function independently at its own inherent rhythm (in this instance 9 to 18 days) and affect changes in the behavior of the organisms through periodic alteration in the amount of secretion poured out circulatory channels not contained in the stalk.

There is not much evidence to support such a conclusion, however. We have no knowledge of any cyclical process inherent in the hypophysis. Indeed that the hypophysis undergoes any cyclical changes at all only recently became known when Smith and Engle (1929), Charipper and Haterius (1930, 1932), Wolfe (1931, 1932) and Wolfe, Cleveland and Campbell (1932) described cycles associated with ovulation. Smith and Engle found that anterior lobes taken from guinea pigs during oestrus had little ovary-stimulating power as compared to those taken during dioestrus. Charipper and Haterius state that the hypophyseal basophiles are much more prominent in the rat in the oestrous phase, while the

eosinophiles predominate during dioestrus. Wolfe has shown that the pituitary of the sow injected into the rabbit is 40 times more potent in precipitating ovulation when active follicles and degenerating corpora lutea are present, than it is in the phase showing active corpora lutea. Wolfe, Cleveland and Campbell found cyclical changes of the dog's hypophysis associated with the oestrous cycle. It is our belief, as will be indicated in greater detail below, that both these cyclical manifestations simply reflect the 4 to 5 day changes in the ovary and do not originate in the hypophysis. But were we to accept the 4 to 5 day cycle as an hypophyseal function it would in no way argue for the presence of a 9 to 18 day rhythm in this gland.

More indirect evidence against this conclusion lies in the wide range of variation, 9 to 18 days, and in the fact that each animal has its own rhythm, 9.6 days in one animal, 13.0 days in another, etc. If the hypophysis were responsible for such a mechanism one would expect a much smaller range of variation and much the same rhythm in all individuals of any given species.

TABLE 1

MULTIPLES OF 4 TO 5 DAY CYCLE	AVERAGE LENGTH OF CYCLES IN DAYS IN PRESENT EXPERIMENTS		
$1 \times 4.5 = 4.5$ days			
$2 \times 4.5 = 9.0$ days	9.6	11.2	
$3 \times 4.5 = 13.5$ days	13.0	14.3	Pseudo-pregnancy (14 days)
$4 \times 4.5 = 18.0$ days	18.4		
$5 \times 4.5 = 22.5$ days			Gestation period (22 days)

Further consideration of the problem led to another theory which seems to bring most of the known facts into line, and at the same time to bring the cyclical phenomena into close relation with the phenomenon of pseudo-pregnancy. According to this theory section of the pituitary stalk modifies the function of the ovaries in such a way that ovulation occurs not every 4 to 5 days as normally but at an even multiple of this interval (see table 1).

In the above table the length of the sex cycle was estimated at an average of 4.5 days. Thus we see that the 9.6 day cycle would approximately equal two of the 4.5 days; the 13.0 and 14.3 day cycles, three 4.5 days, etc. The post-operative rhythm of four of our five animals fits into such a scheme even though the range is as great as 9 to 18 days. The animal with the 11.2 day cycle may appear at first glance as an exception. Inasmuch as some females do show 5 and even 6 day ovulation cycles, however, this one may still be considered as an even multiple of the fundamental rhythm.

The record shown in figure 3 is particularly instructive in this connection. It will be noted that in this animal very striking peaks of activity appeared

after the operation, spaced for the first 60 days in approximately twelve day or six day intervals, and from then on at regular ten or five day intervals. It can be seen here how the longer intervals are definitely multiples of the shorter cycle. Finally the animal ran only regular 5 day cycles. At autopsy it was found that the stalk was partly intact. It is possible that for some time after the operation when the longer cycles were present that the stalk connections were not functioning normally, and that later on as they became normal again, the shorter cycles appeared again, sporadically at first, then permanently.

It would seem, then, that the 4.5 day cycle may persist after pituitary stalk section without giving any outward indication of its presence in vaginal smears or spontaneous activity, except every two, three, or four cycles.

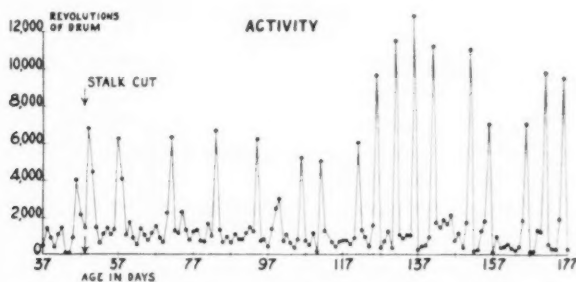


Fig. 3. Activity record of animal in which part of stalk may have become functional again after attempted total section.

In keeping with this assumption are the findings of Loeb (1911), Swezy and Evans (1930), and Nelson (1929) that the ovulation cycle continues within the ovary during pregnancy without giving any indication of its presence in the vaginal smears. Loeb found the ovulation cycle present in the ovary of the pregnant guinea pig, Long and Evans (1922), Swezy and Evans (1930) and Nelson found it in the rat. Swezy and Evans report that at each 4 to 5 day period during pregnancy ripe follicles are present and that at the beginning of each period newly formed corpora are found some of which contained a segmented ovum; and Nelson, as well as Long and Evans found that in some instances the rats actually copulated at this point during pregnancy. With the exception of slight alteration at such times in the typical pregnancy smears there were no other signs of change. Swezy and Evans often found on the fifth day of pregnancy a pro-oestrous smear, especially in the reduction of leucocytes.

Of unquestionable significance in this connection is the fact that in the rat, guinea pig, man and doubtless in many other animals the gestation period is an even multiple of the ovulation cycle. Thus in the rat gestation

TABLE 2

RAT	SEX	DURATION OF ACTIVITY CYCLES			STALK	AUTOPSY FINDINGS PITUITARY			DIABETES INSIPIDUS
		Pre-oper.	Post-oper.	Average		Anterior lobe	Post. lobe	Inter. lobe	
683 (14)	♀	4, 4, 3, 5, 5, 5	12, 17, 13, 14, 15, 14, 15, 13, 13, 13, 17, 16, 14, 14, 14, 14, 14, 14	14.3	Cut	Present	Present	Present	Moderate mo.
78A (54)	♀	4	15, 9, 11, 13, 19, 10, 11, 13, 11, 11, 11, 11, 12, 10, 12, 11, 11, 13, 11, 9	11.2	Cut	Present fairly large	Present fairly large	Present large	Marked per- manent
696 (8)	♀	5, 7, 5, 5	12, 13, 15, 12	13.0	Cut	Present	Present	Present	Marked per- manent
258A (59)	♀		17, 19, 18, 18, 20, 18, 19, 17, 19, 19	18.4	Cut	Present abnor- mal appear- ance very dis- turbed	Present very much dis- turbed	Present	Very slight transitory
162A (60)	♀		8, 7, 7, 10, 10, 10, 10, 11, 10, 13	9.16	Cut	Present but not normal ap- pearance	Present but smaller than usual	Present hyper- trophied	Moderate transitory

lasts 22 days, five times as long as the 4.5 day ovulation cycle (see table 1); in the guinea pig it lasts 64 days, or four times the 16 day ovulation rhythm; and in man the duration of normal pregnancy is ten times the average menstrual period, or 280 days.

Inasmuch as all the cycles described above are related to the fundamental 4.5 day cycle which may be a function of pituitary or ovarian activity, or both, it is important to determine in which of the two glands this cycle has its origin. This question has been discussed in detail by many workers. For the present purposes we have collected what seem to be the essential facts and have concluded from this analysis that the 4.5 day rhythm, and the longer cycles as well, have their origin in the ovaries.

The more obvious bit of evidence favoring this view is the fact that the 4.5 day cycle appears only in the female. If it had its origin in the pituitary gland it would necessarily be present in both sexes. Then, there is the fact that in the ovaries we have a simple basis for a cyclical process of growth and atresia of the follicles, while in the hypophysis no such mechanism exists.

And finally we know that any disturbance in the ovaries such as removal of large parts or extensive traumatization, completely and often permanently abolishes the cycle (Wang and Guttmacher, 1927). It will be recalled that in the present experiments the long cycles were likewise eliminated by ovariectomy.

That Parkes (1927) found a persistence of reproductive cycles in mice whose ovaries were subjected to x-rays to the stage of destruction of all the follicular apparatus and all ova, is not in consonance with the view here expressed. In his cases the regulatory function of the cycle was, however, still dependent upon the ovaries.

What evidence there is in favor of the theory that the cycles have their origin in the pituitary gland may be mentioned in passing. In the first place the function of the ovaries, as well as the testes, depends on the secretion from the pituitary gland since without it they become inactive or even atrophy. And in the second place there is the fact that ovulation can be produced in rabbits by anterior lobe implantation (Smith and Engle, 1927; Zondek and Aschheim, 1927; Bacon, 1930). On the other hand, from observations on spayed and pregnant animals, we know that the ovaries exert a reciprocal effect on the pituitary gland (Engle, 1929; Evans and Simpson, 1929). And injections of oestrin are known to produce profound changes in the hypophysis (Leonard, Meyer and Hisaw, 1930). Moreover, the changes produced in the pituitary gland by anterior lobe extract injection are absent after the ovaries have been removed, thus proving definitely that the changes in the pituitary depend on the mediation of the ovaries (Haterius and Charipper, 1931).

All these facts seen in review seem to indicate that the secretions from

the pituitary gland cause the ovaries to function. The latter glands, however, are so constructed that they can respond only periodically to this stimulus, and thus reciprocally they set up a periodic response in the pituitary. In keeping with this view is the fact that repeated injection of anterior lobe extract into hypophysectomized animals does not produce a state of continuous activity in the ovaries, but an interrupted response (Smith, 1927, 1930).

Moore and Price (1932) have developed a theory of the origin of the reproductive cycle on the basis of a reciprocal relationship between the hypophysis and the ovaries. They believe that the cycles originate in the interaction between these glands, not primarily in the ovaries.

For the present purpose the data at hand may be formulated as follows: due to the section of the stalk the amount of pituitary secretion which gets into the general circulation is decreased, more in some animals than in others. The circulating hormone, then, is not enough to bring about the full response of the ovaries, with ovulation every 4.5 days, but it may be sufficient to produce it every second, third, or fourth cycle.

RELATION OF THE CYCLES TO PSEUDO-PREGNANCY. It is interesting to note that pseudo-pregnancy and our cyclical phenomena have several points in common. In the first place the duration of pseudo-pregnancy varies from 7 to 21 days (Long and Evans, 1922; Slonaker, 1929; Wang, 1923; Shelesnyak, 1931), while the activity cycles observed in the stalk section experiments showed a range of variation of 9 to 18 days. Moreover both phenomena have the same average duration of 14 days (Shelesnyak, 1931). And finally according to the recent work of Vogt (1931, 1933) and Haterius (1933), pseudo-pregnancy also is dependent on changes in the pituitary gland.

It would not seem unlikely, then, that if our theory is sound, the length of pseudo-pregnancy is also a multiple of the fundamental 4 to 5 day cycle. The length of pseudo-pregnancy would be measured by the 4 to 5 day cycle just as the length of time of pregnancy appears to be; and by the same analogy, we would expect to find 4 to 5 day cyclical changes in the ovary in pseudo-pregnancy as in true pregnancy. We are not aware that any observations have ever been made on this point.

In pseudo-pregnancy the length of the period, i.e., the number of 4 to 5 day cycles that are skipped would depend on the intensity of the stimulus applied to the cervix, and reaching the pituitary gland by the sympathetic nervous system (Vogt, 1933; Haterius, 1933; Ball, 1933). With a smaller stimulus only one or two cycles might drop out, while with a stronger stimulus, as many as four or five would be lost, giving a length of 18 or 22 days. Shelesnyak's frequency table indicates a distribution of this kind.

REMARKS. In the above discussion no reference was made to a possible participation of the posterior or intermediate lobes in the production of

these cycles. Attention may be called to the fact that all of these animals showed symptoms of diabetes insipidus, either temporary or permanent, and according to our point of view regarding the origin of this condition, it would follow that the posterior or intermediate lobe must have been involved in the present phenomena.

Nor was any reference made to the brain lesions. We do not wish to minimize in any way the possible importance of the brain lesions. However, the fact that over two hundred animals with lesions in all parts of the tuber cinereum but without stalk injury failed to show this phenomenon is highly significant. It is hoped that these various factors may be evaluated more fully in later experiments.

SUMMARY

1. Five rats in which the pituitary stalk had been sectioned showed extraordinary cycles in spontaneous activity and food- and water-intake.

2. The frequency of the activity cycles was characteristic for each animal, 9.6, 11.2, 13.0, 14.3, and 18.4 days. The group average was 14.0 days.

3. The cycles were characterized by sudden bursts of activity at regular intervals with a relatively low plateau between. Food- and water-intake were the lowest when the activity rise occurred, and highest midway between.

4. This phenomenon appeared only in females. Vaginal smears showed cornified cells on the day the burst of activity occurred and dioestrous smears on the intervening days. Ovariectomy completely eliminated all the cycles in activity and food- and water-intake as well.

5. In an attempt to assign the origin and significance to these cycles, it was pointed out that they probably represent even multiples of the fundamental 4 to 5 day ovulation cycles.

6. The possible relation between the activity cycles produced by section of the pituitary stalk and the phenomenon of pseudo-pregnancy is also probably timed as a multiple of the 4 to 5 day cycle.

7. It was concluded that the cycles originated in the ovaries rather than in the pituitary gland, otherwise the 4 to 5 day cycles would be found in the male as well.

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SURFACE AREA IN A MONKEY, *MACACUS RHEBUS*

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None of the monkeys or apes have as yet been much used in investigations on respiratory metabolism. The desirability, however, of using a primate form other than man for certain experimental work in this field makes necessary the development, not only of proper apparatus and technics of measurement, but also of standards and units of reference for the metabolic data obtained. The expression of the heat production in terms of the first power of the body weight is unsatisfactory whenever there are considerable differences in weight among the individuals used. In the Indian monkey (*Macacus rhesus*), the most available species for laboratory purposes, the range in weight of sexually mature individuals may be of the order of 1 to 4. Pending the development of adequate standards derived from the metabolic data themselves, body surface or some function of body weight may serve empirically to make comparable the results in large and small animals. Surface area, empirical though it admittedly is, has at least common usage in other species, including man, to recommend it, and will perhaps serve until better standards are established (Mitchell, 1930).

In the course of some work with monkeys opportunity was taken to measure the skin areas of six animals, all *Macacus rhesus*, which died or were killed for some purpose. The method used for keeping the skin of the same size as when on the animal was to coat it with a quickly drying, tough and inelastic lacquer (Lee and Clark, 1929). This method is much simpler and easier than making a mold or cast of the animal and allows, we believe, a highly accurate determination of the skin area for the condition and posture of the animal when the lacquer is applied. The range in weight was from 821 to 6600 grams and in age from around seven months to over ten years. Animals II and V whose ages could not be estimated were apparently sexually mature but had not erupted their third molar teeth. Monkey IV was given to us through the courtesy of Dr. A. W. Sellards, Harvard Medical School, and is known to have been more than ten years old. The animals were purposely selected to cover as wide a range in weight, age and nutritive condition as possible.

After death, weight and length measurements were taken and the hair was removed by clipping closely and by depilation in some regions with barium sulfide. The skin was then washed with alcohol and rapidly dried. The carcass was suspended by a hook fastened into the top of the cranium and by the extended arms held with cords. This posture is, of course, not that which would be assumed by a monkey in a metabolism chamber, and undoubtedly tends to give a somewhat larger area than a quietly resting animal presents. It is, however, convenient and reproducible, and eliminates the wrinkles around the neck which cause a variable error in measurements with other postures.

One or usually two coats of a nitro-cellulose lacquer were applied with a brush to the whole surface of the skin and allowed to dry thoroughly. When the lacquer had hardened, the pelt was removed. Particular care had to be taken in skinning the hands, feet, and buttocks, where there was close adherence to the underlying tissues, in order to avoid cracking the varnish and subsequently stretching the skin. The pelt was cut into many small pieces, from 17 to 56, so that it could be spread flat without breaking the lacquer covering. The pieces were first outlined on paper with pencil, then these outlines were traced onto smooth bristol board, and their areas measured with a planimeter. The ears were cut from the pelt at their base, outlined, and the areas doubled to give both surfaces. The area of the tail was measured, not calculated. The measurement of body length used was from the top (crown) of the head to the buttocks, with the dead animal placed back down on a table and straightened with gentle pressure and traction. It corresponds to the sitting height or trunk length used for man.

The data on weight, body length, and the measured skin areas for the six animals are given in table 1. The values of K in the Meeh-Rubner formula

$$S_{sq. cm.} = K \cdot W_{gm.}^{\frac{1}{2}} \quad (\text{formula 1})$$

are also given.

It has been shown for several species of mammals (Dreyer et al., 1912-13; Cowgill and Drabkin, 1927; Lee and Clark, 1929) that other exponents of W than 0.667 give less variable values of K and also closer agreement of measured and calculated areas. We have tested this for the monkeys of the present series by varying the exponent from 0.60 to 0.72, calculating the individual values of K and the coefficient of variation for each mean K . These coefficients of variability for the several exponents are: 0.60 — ± 7.43 per cent; 0.61 — ± 7.00 per cent; 0.64 — ± 6.21 per cent; 0.65 — ± 6.13 per cent; 0.667 — ± 6.05 per cent; 0.68 — ± 6.80 per cent; 0.70 — ± 7.00 per cent; 0.71 — ± 7.50 per cent. Thus, in the simple Meeh-Rubner

expression, the two-thirds power of W gives the best agreement of the data. For this formula with the exponent 0.667, the values of K range from 10.9 to 12.9 and the mean is 11.7. On the basis of this value, the average deviation of calculated from measured areas is ± 5.5 per cent.

Various modifications of the Meeh-Rubner formula for several species have been suggested and used. The introduction of the body length as a variable is common and often gives a definite betterment of the accuracy of

TABLE 1

NUMBER OF MON- KEY	SEX	APPROXIMATE AGE	LENGTH, (CROWN- BUTTOCK)	BODY WEIGHT	MEASURED SKIN AREA	$\frac{S}{K, W^{\frac{2}{3}}}$	NUTRITIVE RATIO, $\frac{W^{\frac{2}{3}}}{L}$	REMARKS
			cm.	grams	sq. cm.			
I	Male	1 year	27.9	1075	1144	10.9	0.367	Immature. In good physical condition
II	Male	Unknown	43.7	2950	2296	11.2	0.328	In fair physical condition
III	Female	9 months	27.5	1072	1179	11.3	0.372	Immature. In good physical condition
IV	Male	10 years	51.5	6600	4042	11.5	0.364	Stocky, heavy body. Considerable subcutaneous and abdominal fat. In good physical condition
V	Female	Unknown	43.2	3540	2871	12.4	0.353	In fair physical condition
VI	Male	7 months	27.1	821	1135	12.9	0.346	Died of pneumonia. In poor physical condition, somewhat emaciated. Had lost most of hair
Mean.....						11.7	0.355	

prediction. Cowgill and Drabkin (1927) used such a modification in their formula for the dog and also showed its applicability to other species. They introduced length as a variable in a form which they termed the "nutritive index," $\frac{W^{\frac{2}{3}}}{L}$, similar in principle to the *pelidisi* of von Pirquet (1917, 1922). For the rat, too, the use of this ratio has been shown to increase the accuracy of prediction (Lee and Clark, 1929). A test was made with the present data on the monkey to determine if the introduction

of body length in this way would give better prediction. The nutritive ratios were calculated and are given in the table. The highest ratio found was 0.372 in monkey III. For a maximal value, 0.39 was chosen and applied in the formula

$$S_{\text{sq. cm.}} = K \cdot W_{\text{gm.}}^{\frac{1}{2}} \cdot \frac{0.39}{\frac{W_{\text{gm.}}^{\frac{1}{2}}}{L_{\text{cm.}}}} \quad (\text{formula 2})$$

The average value of K for this formula is 10.6 with a range from 9.4 to 11.5. It gives only slightly better agreement of calculated and measured areas than formula 1. The average deviation of calculated from measured areas was ± 5.2 per cent, but animal II with the very low ratio of 0.328 gave a difference of $+13.2$ per cent. Without this animal the agreement is significantly better, but with only six animals the superiority of formula 2 cannot be definitely demonstrated. The technical difficulty of obtaining a good length measurement upon a refractory monkey is sure to be considerable. Anesthesia would probably be necessary but undesirable. In view of these considerations, the Meeh-Rubner formula 1 with 11.7 for the value of K would seem to be more usable.

SUMMARY

The skin areas of six *Macacus rhesus* monkeys were determined. The skin on the animal was coated with a quickly drying, tough lacquer, then removed, cut into pieces and the outlines of the pieces measured with a planimeter. It was found that 0.667 as the exponent of W in a formula of the Meeh-Rubner type gave the best agreement of calculated with measured areas. The mean value of K in the formula $S = K \cdot W^{\frac{1}{2}}$ is 11.7, with an average deviation of ± 5.4 per cent.

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MECHANICAL FACTORS CONTRIBUTING TO THE EXCHANGE OF FLUIDS IN THE BODY

V. EXCHANGE DUE TO MOVEMENTS OF SYSTEMIC ARTERIES AT CARDIAC SYSTOLE AND DIASTOLE

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The individual arterial anastomoses of arterial networks are herein called arteries similarly as the individual capillary anastomoses of capillary networks are commonly called capillaries. Even the "descending thoracic aorta" is a collective name applied to a series of arteries or arterial anastomoses of considerable size that connect the aortic ends of the relatively small "aortic intercostal arteries" and some other small arterial anastomoses with one another. The arteries, like the capillaries, are so numerous that it is probably not possible to name all of them or even to count all of them. The usual collective names for arteries will be used, therefore, but this will be done as a mere matter of convenience in speaking and writing. Our considering arterial anastomoses as arteries serves the important purpose of placing a needed emphasis on the fact that the systemic arterial system is very much unlike a tree (1930). This system exists essentially as networks of arteries in the skin, the skeletal muscles, the bones, the eye, the central nervous system, the heart, the liver, the spleen, the walls of the gall and urinary bladders, the trachea, the esophagus, the stomach, the intestines and even the kidneys of many mammals. My injected and cleared specimens show these networks clearly.

Terminal or end-arteries are also present in the body. These are arterio-capillary anastomoses in the sense that they connect arteries of arterial networks with capillaries of capillary networks. A few of these end-arteries, e.g., the renal artery in some mammals, are quite large, but the greater number of them throughout the body are very small. Blood is normally drained from the greater number of the arterial anastomoses in the body through the special drainage channels herein referred to as end-arteries. In the walls of the ventricles of the normal heart (of the human being, tiger, lion, bear, dog or hyena) there are probably not less than fifty thousand and probably not more than one hundred thousand large and small arterial anastomoses from which blood flows into the capillaries through

a larger number of extremely small end-arteries. These are not end-arteries in the sense of Cohnheim. They are the extremely small arteries that originate from the numerous anastomoses of the network of arteries. They are the arterio-capillary anastomoses. There is probably no end-artery in the sense of Cohnheim in the walls of the ventricles of the heart.

Besides the arterial anastomoses and the arterio-capillary anastomoses probably the only other individual arteries found in the body are the vasa efferentia in the kidney. These might be referred to as glomerulo-capillary anastomoses.

Numerous systemic arteries of such an animal as the dog normally do something in response to the extra volume of blood thrown into them at cardiac systole. They exhibit pulsatile changes of which the most conspicuous feature is systolic arterial looping. This looping is generally accompanied by an increase but in instances by a decrease in the length of the arteries. Some arteries normally elongate at systole without looping, some become tortuous by forming arciform loops and some arteries which are already looped at diastole loop still more at systole. All of these arteries return to their diastolic positions and lengths after systole ends. They are moving during the greater part of each normal cardiac cycle, and it is technically very difficult, therefore, to determine whether a change in diameter is an essential part of a pulsatile change or whether a change in diameter normally occurs in numerous arteries during the cardiac cycle.

There are many arteries in the body which normally loop very little, if at all, at cardiac systole. The greater number of these might be considered as reserve arteries in the sense that they do loop under certain conditions of circulation, as in normal old age. Many of the reserve arteries are very small but some of them are quite large. The end-arteries have so rarely been observed to loop at systole that they are not considered as reserve arteries. The reserve arteries are arterial anastomoses which are distributed among the other arterial anastomoses of networks of arteries.

Figures 1, 2 and 3 show many arteries. Many of these are reserve arteries. Under certain conditions of circulation arteries of all such networks undoubtedly loop at cardiac systole. I have seen them loop on the surface of the brain, in Glisson's capsule, in the capsule of the spleen, in the muscles of the chest walls, in the ocular muscles and in various other parts of the bodies of various animals. The systolic loops are often quite small, but they would have to be extremely small to fail to contribute to the exchange of body fluids.

A physiological significance of the lateral to and fro movements of arteries is offered by Tannenberg and Fischer-Wasels (1927). They presume that the rhythmically looping arteries compress veins at each cardiac

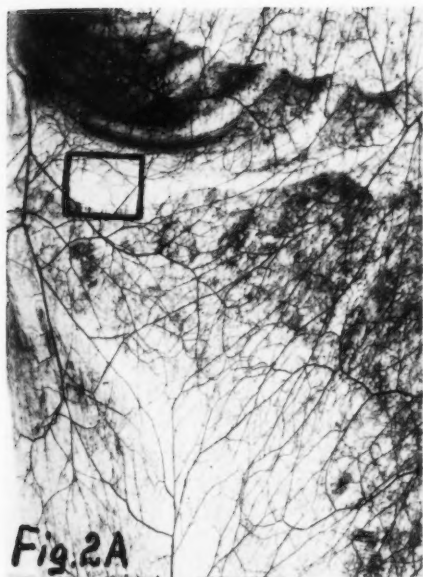


Fig. 2A



Fig. 2B

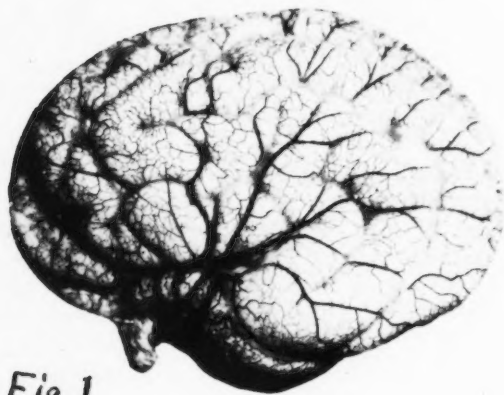


Fig. 1

Fig. 1. Cerebral hemisphere of a 139 day old tiger. Arteries only. Injected with India ink. The greater number of the arteries on the surface of the brain and in the substance of the brain are reserve arteries. Systolic looping of all of them occurs in extreme old age if the heart beat is slow and strong. In younger animals many of these arteries rarely loop at cardiac systole. It is clear from this photograph that in order to talk about or count the arteries accurately one must consider each individual arterial anastomosis as an artery.

Fig. 2A. Part of chest wall, part of abdominal wall and part of diaphragm of a 139 day old tiger. Arteries only. Injected with India ink. Under normal conditions of circulation the greater number of the arteries that are most clearly seen in the photograph loop at cardiac systole.

Fig. 2B. Enlarged portion of figure 2A. Taken from the relatively clear rectangular area marked off with pen and ink in figure 2A. These are principally reserve arteries that undergo systolic looping in normal old age and under certain unusual conditions of circulation in younger animals. A slow heart and a large volume stroke favor systolic looping of such reserve arteries.

systole and thereby contribute to the flow of venous blood to the heart. These authors give Hasebroek (1914) credit for the idea, but I fail to find that Hasebroek mentions the looping or lateral swinging of arteries in the work cited. However, numerous arteries lie alongside at least one vein, and arteries often pass somewhat at right angles to and immediately under or over veins. Many arteries have little room to loop except into the veins. The sudden systolic looping of arteries disturbs the nearby veins and, by the same act, temporarily retards the circulation of the venous blood.

The augmented flow of venous blood toward the right heart is probably not more important than the simultaneous damming back of the venous



Fig. 3. From abdominal wall of a very old Buttikofer's guenon. This monkey was probably twenty-six years of age. Arteries only. Injected with India ink. Note the tortuosity of the arteries and that the very small arteries, as well as the largest ones, meander. The reserve arteries probably ceased to be reserve arteries several years before the animal died.

blood on the capillary side of the loops. This systolic damming back of the venous blood should increase the filtration pressure in the capillaries during a short part of each cardiac cycle and cause some of the fluid of the blood to escape from the blood stream through the capillary walls. Some of the intercapillary fluid should then filter back into the capillaries at a later time and for a relatively long time during the cardiac cycle, i.e., after the pressure on the veins is released and the intercapillary pressure accordingly exceeds the intracapillary pressure. It is interesting to note that the increase in the capillary pressure due to the partial and total occlusion of veins is coincident with the increase in capillary pressure due to the systolic increase in the arterial blood pressure. During the remainder of the cardiac cycle, usually about five-eighths of it when the circulation

is normal, the blood pressure diminishes on both sides of the capillaries simultaneously. The capillary blood that is affected by the partial or total occlusion of veins should accordingly show pulsatile changes in pressure but not pulsatile changes in velocity of flow. At points where microscopic examination of the blood in the capillaries reveals no pulsatile velocity changes pulsatile changes in pressure can invariably be determined by cannulating one of the capillaries; a water column supported by the intracapillary pressure invariably falls and rises during each cardiac cycle whenever the systolic looping of arteries is normal or about normal. When the systemic arteries do not loop, as under various pathological conditions, the veins are not partially or totally occluded at each systole and pulsatile velocity changes can be observed in the capillaries and often in the small veins.

The passing back and forth of liquid through the capillary walls should permit a better exchange of respiratory gases and of nutrient and waste substances in general than could possibly occur if the arteries merely dilated at systole. Rhythmic contractions of the muscles of the body should of course compress veins and accordingly cause an exchange of fluids through the capillary walls, but there are occasions when the skeletal muscles are somewhat inactive for long periods of time, as in normal sleep. It is perhaps a fortunate circumstance, therefore, that the arteries loop during relative inactivity of the body somewhat as they do at other times. It is probable that the arterial looping is often greater during sleep than at other times. In some people this is at least true of the radial artery and certain others that can be observed through the skin.

The arteries that loop should periodically compress many of the lymph vessels, similarly as they compress veins, and should likewise augment and impede the circulation of lymph.

If the reasoning already presented is correct we can speak of a mechanical exchange of body fluids that is brought about by the systolic pounding and deforming of veins and lymph vessels. A further and perhaps a more important mechanical exchange of fluids is probably brought about by the systolic and diastolic compression and decompression of intercapillary and intracapillary fluids in the immediate vicinity of each systolic arterial loop. The systolic looping of the arteries should be expected to mobilize the water in the cells and intercellular spaces and to periodically cause relatively high pressure areas in the tissues and thereby contribute to the flow of liquid in the intercapillary spaces, through cell walls and through capillary walls.

When an artery loops, as in figure 4S, the heterogeneous tissue about it is unequally compressed and decompressed above the artery and unequally decompressed below it. Liquid must flow from at least some of the relatively high pressure areas to at least some of the relatively low pressure

areas in the compressed and decompressed tissue when the arterial loop forms at cardiac systole. At diastole the directions of flow of the liquid are reversed. However, liquid flows so sluggishly in the intercapillary spaces and the blood and lymph capillaries are so much more inflatable than anything else in the tissue that at cardiac systole the tissue fluid probably flows in larger amounts from even the most highly decompressed intercapillary space into the neighboring capillaries than from one intercapillary space into another. At diastole the flow is principally from the capillaries into the intercapillary spaces because the capillaries are so much more decompressible or deflatable than the tissue outside the capillaries. The compression and decompression of the heterogeneous tissue must occur very quickly, as they actually occur at diastole and systole, in order for this mechanical exchange of fluids to take place; otherwise enough blood

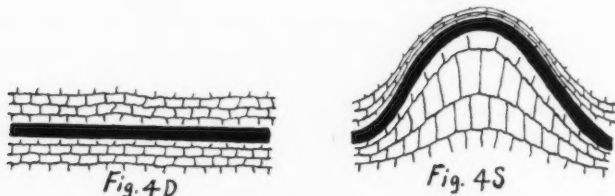


Fig. 4D. Representation of an artery imbedded in capillaries and as being in its diastolic position. One hundred and forty-eight capillaries are arbitrarily placed above and the same number below the artery.

Fig. 4S. The same artery and capillaries, as in 4D, represented here in their systolic positions. Note that the ratio of the linearly inflated capillaries to the linearly deflated ones is approximately four to one. The extent of the looping of the artery is exaggerated for the sake of clearness.

would have ample time to flow out of or into the two ends of any capillary (from distant parts of the capillary network) to permit it to deflate or inflate without extracting from or giving liquid up to the intercapillary spaces.

It is important to observe that a much greater number of capillaries elongate than shorten at systole when capillaries are equally distributed on all sides of an artery that loops. In figure 4D, 148 capillaries are arbitrarily placed above and the same number below the artery. Figure 4S shows that at systole all of the capillaries below the artery are stretched, that 90 of the capillaries above the artery are stretched and that 58 of the capillaries above the artery are shortened. According to figure 4S the ratio of the capillaries that lengthen to those that shorten is about four to one. It is important to note that very frequently in the body there is an unequal distribution of capillaries above and below, i.e., on the different sides of the arteries that loop. In many instances arteries rest on a bed or network

of capillaries at diastole, and these arteries generally loop away from these capillaries without looping into others. This is essentially true, also, when an artery loops into a vein. In such instances the ratio of the capillaries that elongate to those that shorten at cardiac systole is many times greater than four to one. At cardiac diastole the arterial loop diminishes in size or even disappears completely, and the capillaries that were stretched at systole become shorter while those that were shortened at systole become longer. If liquid flows principally from the intercapillary spaces into the systolically elongating and elongated capillaries it must flow principally from the diastolically shortening and shortened capillaries into the intercapillary spaces. This would mean a much greater mechanical exchange of fluids than could possibly occur if the arteries merely dilated at systole and constricted at diastole.

In order to determine the visible effects of the arterial looping on the capillary circulation, and accordingly the probable effects of the looping on the exchange of fluids through the capillary walls, a large number of observations of capillaries at various parts of the body were made with a binocular microscope under different conditions of illumination when the tissues were stretched strongly, mildly, rapidly and slowly. Different methods were employed to stretch the tissues. Artificial stretching was resorted to only after many attempts to observe the circulation in capillaries in the immediate vicinity of systolically looping arteries proved futile; any of the loops developed so quickly at systole and died down so rapidly at diastole that it was not well possible to determine what changes took place in the capillaries. Probably the most trustworthy results were obtained by artificially stretching the networks of capillaries in many directions simultaneously, i.e., by simulating the normal stretching, as represented below the artery in figure 4S. This was done by placing a kangaroo tendon of appropriate size through an artery, properly ligating the artery to prevent bleeding, making one end of the tendon stationary by clamping it and then pushing the other end until the tendon looped and carried the artery with it. The direction of the looping was generally predetermined by scraping one side or by making many shallow cuts across one side of the tendon. This procedure was followed in bringing about looping of various arteries in a large number of dead zoo animals and of anesthetized dogs, cats and rabbits.

As the capillaries became longer, as pictured below the artery in figure 4S, the lumina that changed in any way became either smaller or larger when the tissue was stretched about as mildly as it is normally stretched by the largest arterial loops that develop at systole in a dog of average size, and blood flowed from the relatively unstretched into the more strongly stretched capillaries. It is quite safe to conclude from the observations that when a capillary, like a rubber tube with porous walls,

is subjected to mere linear extension by pulling the ends, a demonstrable increase in the volume capacity occurs even when the lumen becomes smaller; the increase in length more than offsets the decrease in the size of the lumen. The lumina as well as the lengths of some capillaries were observed to become greater when the tissue was stretched. It is obvious that the volume capacity of each of these capillaries increased at systole and decreased at diastole.

Observations were made on capillaries in tissue that was subjected to greater artificial stretching than occurs as a result of the strongest systolic looping of arteries, but these observations are not considered here. The chief reason for omitting them is that the tissue was in many cases unquestionably heterogeneous in the sense that it consisted of fibers and cells of different extensibilities, different elasticities, different lengths and somewhat irregular connections. Effects of the heterogeneity become apparent only upon stretching the tissue abnormally strongly. It is only then that fibers of little extensibility become maximally extended before others and cause undesirable effects such as the occlusion of capillaries without permitting them to open up until the arterial loop becomes smaller. As a rule the extensibility and other qualities of the tissue elements are apparently great enough that when a heterogeneous tissue is stretched only mildly it behaves essentially as a homogeneous mass that has a dense network of inflatable capillaries in it.

The volume capacity of a capillary is greater when moderately stretched than when not stretched, especially if it is stretched laterally as well as linearly. In the sense that the volume capacity increases, a capillary inflates when it is moderately stretched, even in case the lumen grows somewhat smaller. This is linear inflation which is possible only under the condition that liquid flows into it to permit the inflation, for we cannot well presume that either the blood or the tissue fluid effervesces as a result of the mild decompression. The liquid that permits the inflation of a capillary may enter the vessel from one or both ends as well as through its walls.

If the stretching of a capillary occurs very slowly the required amount of liquid to permit the inflation flows in from one or both ends, but if the rate of stretching is very great, as it is at cardiac systole, the liquid required for the inflation comes principally through the capillary walls from the intercapillary spaces before the blood can find its way through one or more of the many other inflated capillaries of the capillary network to flow into this one through one or both ends. This means systolic dehydration of the tissues surrounding the capillary.

At diastole the volume capacity of the capillary diminishes, as the capillary deflates, provided some liquid leaves through one or both ends or passes through the capillary walls into the intercapillary spaces. If

the linear deflation of the capillary at diastole should take place as rapidly as the linear inflation of it occurs at systole, there would be a diastolic hydration of the tissues equal or almost equal in amount to the systolic dehydration of them. However, the arterial loops develop much more rapidly at systole than they die down at diastole. Therefore, the capillaries stretch much more rapidly at systole than they shorten at diastole, and the systolic dehydration should accordingly exceed the diastolic hydration in amount.

Direct observation of the flow of blood in mechanically stretching and stretched capillaries shows that the blood cells in the capillaries often behave as one-way valves and in such a manner that the systolic dehydration should be expected to exceed the diastolic hydration of the tissues still more than has been explained. Upon stretching the capillaries in many different directions at the same time (as under the artery in fig. 4S) the lumina of some of the capillaries become so small that they appear to be securely blocked here and there for a very short time by erythrocytes. These cells dam back the liquid of the blood from one or both ends and thereby permit an unusual amount of intercapillary fluid to enter at least some of the capillaries of the network. Upon the entrance of a certain amount of the intercapillary liquid into the blocked capillaries the lumina of these capillaries become large enough that the erythrocytes circulate again. The capillaries are not blocked at diastole by the erythrocytes. This valve-like action of the erythrocytes further increases the mechanical hydration-dehydration difference in favor of systolic dehydration of the tissues in the neighborhood of the systolic arterial loops.

SUMMARY AND CONCLUSIONS

1. Under normal conditions of circulation the most conspicuous feature of the pulsatile movements of systemic arteries is systolic arterial looping.
2. Some arteries loop into veins. This drives some of the venous blood toward the heart. It also dams back some of the venous blood on the capillary side of the loops and accordingly increases first the intracapillary pressure and then the intercapillary pressure as liquid filters through the walls of the capillaries into the intercapillary spaces. As soon as systole ends, the intracapillary pressure falls and some intracapillary liquid filters back into the capillaries. There is, therefore, a greater exchange of respiratory gases and nutrient and waste substances in general than could take place if the arteries merely dilated at systole and constricted at diastole.
3. Capillaries are inflated and deflated in the vicinity of each arterial loop. As a result of the looping of an artery a greater number of the capillaries become inflated (linearly and laterally) than become deflated. The reverse occurs, but more slowly, as the loop dies down relatively slowly at diastole. This difference in the rate of inflation and deflation

of the capillaries that are inflated at systole is responsible for a hydration-dehydration difference in favor of systolic dehydration of the tissues in the vicinity of each arterial loop.

4. The hydration-dehydration difference is often increased as a result of a blocking, by erythrocytes, of the capillaries which are inflated linearly during systole.

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MECHANICAL FACTORS CONTRIBUTING TO THE EXCHANGE OF FLUIDS IN THE BODY

VI. EDEMA DUE TO THE FAILURE OF SYSTEMIC ARTERIES TO LOOP AT CARDIAC SYSTOLE

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The pulmonary artery and its branches, unlike many systemic arteries, do not loop at cardiac systole. The pulmonary branches can often be observed to dilate slightly at systole. These statements apply to the lungs of birds and mammals. The bird lung, unlike the mammalian lung, does not inflate at inspiration, and it fails to become edematous under normal conditions only because there are some special drainage channels through which liquid is mechanically deflected from the respiratory parenchyma into the lower thoracic aorta (1931c). This liquid is a filtrate from the blood, and it becomes excessive in amount in the lung tissue of the bird whenever the canals of the special drainage system are blocked, as sometimes occurs in certain mycotic infections. When the mammalian lung is as completely demobilized as the lung of the bird normally is, it becomes edematous because the liquid that filters through the capillary walls into the intercapillary spaces and alveoli is not drained away in sufficient amounts through the lymph channels to prevent the edema. Under normal conditions of breathing of the mammal, liquid of the blood filters into the intercapillary spaces during expiration and is then mechanically returned to the blood in the pulmonary capillaries during inspiration (1930b). Elsewhere in the body there is a comparable mechanical deflection of liquid from the intercapillary spaces as the result of piezometric deflection (1930a) and, as pointed out in the preceding paper, of the systolic looping of systemic arteries.

Such observations (together with a number of other similar ones that can be made on reptiles, fishes and certain other animals) seem to show that normal tissue ordinarily remains normal under the condition that at least one type of mechanical force is always at play to not only provide for a greater exchange of body fluids than could otherwise occur but also to prevent edema. Systolic looping of numerous systemic arteries is essential

for the normal exchange of fluids through the walls of the systemic capillaries of mammals and birds. It is quite evident that the amount of this looping must vary inversely with the amount of systolic dilatation of the arteries because the extra volume of blood entering the aorta at systole can be accommodated as a result of linear extension primarily, of lateral extension primarily or of various combinations of linear and lateral extension of the systemic arteries. Edema occurs, according to my observations, whenever the arteries dilate so readily at cardiac systole that the systolic looping is insignificant or absent. The edema is the direct result of the absence of the looping, and it is the indirect result, therefore, of the systolic dilatation of the arteries. In order to study the causal connections existing between edema and the behavior of systemic arteries it becomes essential to inquire into the relative amounts of looping and dilatation of the arteries under both normal and pathological conditions of circulation.

The cardiac end of the ascending thoracic aorta of an apparently normal dog that weighed 12.4 kgm. moved three or four millimeters toward the diaphragm, the aortic arch straightened a little and a point at the apex of the arch moved two or three millimeters toward the head at each cardiac systole when a left-sided pneumothorax existed for the purpose of making the observations and then the animal was in ether anesthesia and was given artificial respiration some of the time. A conservative estimate of the extra amount of blood accommodated as the result of the systolic elongation of the thoracic aorta was 0.3 cc. On the basis of measurements and estimates of the amounts of systolic elongation of various other arteries of anesthetized dogs it was judged that the larger arteries of the neck and front legs of the 12.4 kgm. dog elongated sufficiently for them to accommodate 1.0 cc. and that the systolic elongation of arteries in the abdominal cavity was adequate to permit these to accommodate at least 2.5 cc. more of the left ventricular output of blood. The numerous other arteries of the body that normally undergo systolic elongation could easily accommodate 3.0 cc. of the volume stroke of the left ventricle. If these figures are correct the arteries of a 12.4 kgm. dog elongate sufficiently at each normal cardiac systole to accommodate 6.8 cc. of the left ventricular output without dilating.

Immediately after the 12.4 kgm. dog was killed the heart was excised, the atria were cut away and the ventricles were suspended by means of a hemostat that was clamped onto some tissue at the basal portion of the interventricular septum. About 12.0 cc. of water were then required to fill either the right or the left ventricle. Since this figure seems to be about correct, in view of various determinations of the volume stroke, let us assume that the normal volume stroke of the left ventricle of this heart was 12.0 cc. of blood. This would mean that the accommodation of 5.2 cc. or about 43 per cent of the blood thrown into the systemic arteries at

cardiac systole remains to be explained. This amount might be expected to cause arterial dilatation of sufficient magnitude to be unmistakably seen with a microscope, if not with the unaided eye, in spite of the technical difficulties in the way of making the observation of arterial dilatation when arterial looping occurs also. But this amount must be greatly reduced.

Before and after the pneumothorax was created the ejection period of the heart generally amounted to about three-eighths of the cardiac cycle, as is often the case in animals. Now if the left ventricular volume stroke of such an animal is 12.0 cc. when the circulation is normal, 12.0 cc. of blood escape from the arteries through the capillaries during each cardiac cycle. If we assume a constant rate of escape of blood from the arterial to the venous side during all parts of the cycle, 1.5 cc. of blood must escape from the arteries through the capillaries during each eighth of the cycle. This would mean that 4.5 cc. of blood must escape during the ejection period of the heart and 7.5 cc. during the remaining five-eighths of the cycle. Since the disposition of only 5.2 cc. of the left ventricular output remained to be accounted for and since 4.5 cc. escape through the capillaries during the ejection period of the heart, the accommodation of only 0.7 cc. of the left ventricular volume stroke now remains to be explained. It is of course preposterous, in view of the technical difficulties involved in making observations of systolic arterial dilatation, to assume that 0.7 cc. of blood (or even two or three times this amount) can cause an arterial dilatation of sufficient magnitude in a 12.4 kilogram dog to be unmistakably detected with the unaided eye unless the dilatation is quite local, perhaps limited almost entirely to the aorta.

Some x-ray photographs obtained by Bickenbach (1931) are of interest at this time. These are records of some of the systolic and diastolic reactions of the ascending thoracic aorta of the normal human being. The method consists essentially of photographing the aorta through narrow horizontal slits between descending (falling) lead plates. Due to different movements of the ascending aorta during each cardiac cycle the technical difficulties associated with the method might seem to be great, as Bickenbach admits in part, but it is well possible that at least some of the apparent dilatation recorded means actual systolic dilatation.

The ascending thoracic aorta of the rabbit appears to behave in essentially the same ways as does that of the dog. A dilatogram obtained from this aorta of the rabbit by Broemser and Ranke (1930) shows a greater systolic dilatation than I have been able to detect in any large or small rabbit except when this aorta was injured by scraping or crushing. It should be noted in this connection that Broemser and Ranke injected *hirudin* and urethane intravenously, that they opened the chest under artificial respiration, that they dissected the ascending aorta free from the tissues about it, that they passed a cannula of a manometer down a common

carotid artery and into the ascending aorta, that they connected another manometer with an external iliac artery and that they placed a dilatograph of two grams' pressure on the ascending aorta. It cannot be claimed that the circulation of such an experimental animal is reasonably normal.

In view of my own observations and certain results in the literature, especially results like those obtained by Bickenbach, I am willing to assume that a systolic arterial dilatation occurs in such an animal as the dog under entirely normal conditions of circulation. My observations were made on anesthetized animals. The arteries were generally observed with a binocular microscope focused on the edges of a narrow slit in thin metal that was placed immediately above or in cases to one side of an artery. The slit was placed at right angles to the long axis of the artery. A fine scale along one edge of the slit enabled one to determine the amount of lateral expansion of the artery, although the lateral movements of the entire artery at systole often interfered with making accurate readings. In some instances a considerable portion of the left chest wall was removed, in others the sternum and costal cartilages were cut away. Artificial respiration was given either all or a part of the time whenever a pneumothorax existed. In still other cases the abdomen only was opened or such arteries as the common carotids, the femoral arteries or the radial arteries were exposed without dissecting them free of the tissues about them except to the extent that this was necessary to make the vessels distinctly visible. These animals were of course not normal, but attention was given especially to the observations that were made when the circulation appeared to be most normal.

The reactions of the arteries varied considerably in puppies that ranged in age from about three to six weeks. It is probably safe to state that the lateral expansion of the arteries of such puppies is great enough for the accommodation of as much as ten per cent and certainly not more than twenty per cent of the total volume stroke of the left ventricle. The ascending aorta dilates most strongly, perhaps because it is here that the forcefully ejected blood from the left ventricle first meets the relatively stationary blood in the aorta. In some of the animals the innominate artery dilated slightly at systole. In the young dogs with closed chests a slight systolic dilatation of the relatively thin-walled abdominal aorta and the common iliac arteries was generally observed. When the circulation is normal or about normal the femoral arteries probably do not dilate at systole unless the animal is on its back and the hind legs are extended more than they normally are in ordinary life. The common carotid and radial arteries were not observed to undergo systolic lateral expansion when the circulation seemed to be quite normal. A slight systolic dilatation of the ascending thoracic aorta (and possibly a part of the cephalic end of the descending thoracic aorta), all of the abdominal aorta, the common iliac arteries and the innominate artery is probably normal for puppies.

Observations made on adult dogs show considerable variations which seem to be independent of sex, breed, age or other definable conditions. In a few of these dogs no systolic arterial dilatation could be observed except at the ascending thoracic aorta while in a few others the dilatation was great enough for the accommodation of at least fifteen per cent of the left ventricular volume stroke when the circulation seemed to be as normal as one could reasonably hope for under the conditions as described in connection with the work on the puppies.

If, as previously stated, 7.5 cc. of blood escape from the arteries through the capillaries between the heart beats of a 12.4 kgm. dog, the arteries must be in such a state of linear and lateral extension at the end of systole that they contain this extra amount of blood. Perhaps the elastic recoil of the elongated arteries is partly responsible for the reasonably high diastolic pressure, but this cannot be a very important factor because, excepting the aorta and some of its largest branches, the linear elasticity of the arteries is almost negligible unless they are stretched considerably more than can occur in the body. The really important factor contributing to a high diastolic blood pressure is the elastic recoil of the systolically deformed tissues in which the arteries are imbedded. The heart, at systole, deforms these tissues by increasing the lengths of the columns of arterial blood in them. As soon as systole ends (allowing a certain time for the transmission of the pressure wave) the deformed tissues return to their normal diastolic positions as rapidly as they can force the arteries imbedded in them to shorten.

If it is the elastic tissues about the arteries—rather than the tissues in the walls of the arteries—that do the major part of the work of propelling the arterial blood between heart beats, we can readily understand why it is not disastrous for the arteries to lose their intrinsic linear elasticity while their linear extensibility decreases only a little. This rather paradoxical situation comes into existence as an animal grows older and is regularly found in normal old age. Typical pictures of such arteries are the well-known meandering or otherwise tortuous arteries of some very old people and animals. Such arteries have extensibility only or primarily in the sense that the tissues about them are still able to extend when the systolic looping of the arteries forces them to extend. They have little or no elasticity of their own, but the deformed tissues about them force them to return to their diastolic positions so that extension can again occur at the next systole. Failure, for any reason, of the arteries to undergo linear extension at systole leads to grave pathological conditions such as edema and hemorrhages. In some instances it is not the arteries as much as it is the tissues about the arteries that are responsible for circulatory accidents and abnormal kinds of circulation.

If an artery that normally elongates at systole is pulled so far out of its normal diastolic position that little or no systolic elongation of it takes place

the chief technical difficulty in observing any change in the size of the vessel is thereby partly or entirely eliminated, but even then a systolic increase in the diameter of the artery may not be perceptible except in case of a skipped beat or some other comparable upset of the circulation. In case of a skipped beat, among normal heart beats, the loss of blood from the arterial to the venous side is great enough that the normal diastolic dilatation of the artery is temporarily lost. At the next heart beat the normal diastolic dilatation (which is also the normal systolic dilatation) is reached and maintained quite well during all parts of the ensuing cardiac cycles unless another beat is dropped, or the heart begins to beat slowly and strongly or the artery is injured in some way as by scraping, crushing, cooling with ethyl chlorid or drying and then moistening.

Generally speaking, there is a critical diastolic pressure and a critical systolic pressure for normal arteries. The critical diastolic pressure is somewhat subdiastolic or subminimal and the critical systolic pressure is somewhat suprasystolic or supramaximal. The maximal and minimal pressures can fluctuate between the upper and lower critical pressures without permitting a perceptible change in the diameter of the arteries at systole. That is, the pulse pressure can fluctuate considerably without permitting the arteries to change perceptibly in diameter, unless the critical pressures are violated as when the heart beats are abnormally slow and strong. One or both of the critical pressures appear to be violated for some arteries much sooner than for others whenever the mean pressure falls or rises. This is especially true of the aorta, the renal artery and the major annulus in the iris. The ascending thoracic aorta even seems to dilate at cardiac systole and to constrict at cardiac diastole when the circulation is normal. The circular muscle fibers in the walls of the greater number of arteries in the body are powerful enough that these arteries offer considerably less resistance to being extended linearly than laterally at cardiac systole.

It is generally considered that the aorta and the other large arteries are normally under tension of such a nature that they are extended linearly. This is especially true of the arteries of a dead animal. As evidence of this the central and peripheral stumps of an artery separate considerably and rapidly as soon as the artery is cut. The systolic elongation of arteries means a further, temporary elongation of the arteries that are already extended linearly.

It is important that we distinguish sharply between systolic reactions of normal arteries and the characteristic systolic reactions of mechanically deformed walls of otherwise normal arteries. An ordinary sphygmogram, as from a radial artery, is by no means a record of a systolic increase in the diameter of a normal artery. Instead, it is a record of certain movements of a mechanically deformed wall of an artery. A good sphygmogram is a record obtained from an artery at the place where the vessel is mashed due

to the action upon it of an external force. The sphygmogram is often very good when the external pressure used to deform the artery is equal to the internal pressure at diastole.

Likewise, when the fingers are placed on a radial artery strongly enough to feel a good pulsatile change in the blood pressure it is not a systolic dilatation of the normal artery that is felt, it is usually the systolic movements of the arterial walls that have been deformed by the fingers. If the fingers are placed too lightly on the skin to deform the artery the pulse can sometimes be detected as the artery, in elongating, jumps quickly to one side or the other or up against the skin. In some of my attempts to record the pulse by placing a very light, pointed rider on a ramus volaris superficialis, the graphic records showed in some cases a fall instead of a rise at systole. Inspection showed that in such instances the artery merely moved to one side at systole and permitted the rider to fall.

In some respects the physiological effects of the systolic elongation and the diastolic shortening of the arteries are very much the same as if the arteries merely dilated at systole and constricted at diastole. Either situation alone or both together contribute to the existence of the pulses with their characteristic features and to a fairly constant flow of blood in the capillaries and the smallest arteries. However, there are some very important consequences of the systolic and diastolic changes in length which could not well exist if the arteries merely dilated at cardiac systole and constricted at cardiac diastole. These consequences were considered in part in the preceding paper, in which the concept of the mechanical hydration-dehydration difference in favor of a mechanical dehydration of the extravascular tissues was developed.

The concept of the hydration-dehydration difference in favor of systolic dehydration of the tissues in the vicinity of systolic arterial loops may seem unwieldy and somewhat out of harmony with the usual ideas that filtration of liquid from the capillaries into the intercapillary spaces occurs generally in the body and that this is normally offset by certain factors, the principal compensatory factor being a greater osmotic flow of liquid into the capillaries than out of them into the intercapillary spaces. It seems to be generally the case that whenever the arterial looping is either very weak or entirely absent tissues become edematous although the blood colloids remain sufficiently normal to prevent the edema if they serve this purpose under normal conditions of circulation. With the development of oligemia the concentration of the blood colloids may even increase while the tissues become more and more edematous. When the heart rate is so great that the arterial loops are about the same at systole as at diastole the edema that develops may be checked or even diminished by increasing the osmotic pressure of the blood, but slowing the heart and permitting a greater volume stroke and accordingly a greater systolic looping of the arteries by adminis-

tering digitalis is a much more effective measure than altering the osmotic pressure of the blood. Mechanical edema resulting from the absence of arterial looping can be combated by mechanical means unless the tissues have already become too edematous to permit the arteries to loop again.

Edema that develops as a result of the failure of arteries to loop at cardiac systole might be called mechanical edema to distinguish it from chemical edema. It is probable that mechanical edema may exist alone, but not for a great length of time; when it develops, the tissues suffer to such an extent that chemical edema follows as a result of osmosis unless the mechanical edema is checked in time, perhaps by giving digitalis. If chemical edema develops first, mechanical edema soon follows because the tissues about the arteries lose their capacity of being extended by the arteries and the arterial looping fails to occur. Even those arteries that normally loop into veins cannot now pull the necessary amount of tissue with them to permit normal looping. In such cases, as in uremic patients, intravenous injections of glucose or magnesium sulphate often serve to reduce the edema sufficiently by chemical means to permit the arteries to loop at systole and reduce the edema still more. The action of the heart often changes in such a manner after these injections that especially strong arterial looping should tend to occur. However, the decrease in the intracranial tension and the beneficial effects produced elsewhere in the body are only temporary because the original cause of the edema continues to exist.

Gum of acacia may be given intravenously to a normal animal to increase the viscosity and incidentally the osmotic pressure of the blood. Observation shows that the arterial looping becomes more prominent, that the rate of growth of the loops increases and that the loops die down more slowly than under normal conditions. The blood, being now more viscous, is more sluggish in its movements through the capillaries and other small vessels. This sluggishness in the forward movement of the blood favors not only a quicker formation of the loops, larger loops and a slower dying down of the loops at diastole but also greater piezometric deflections (1930a) at various points in the body. These conditions, together with the increased osmotic pressure, should favor a greater dehydration of the tissues than occurs under normal conditions.

The increase in the piezometric deflection can be readily understood if we assume a still more viscous blood, such as a contracted blood clot in the aorta and the aortic intercostal arteries. If this clot is either pushed or pulled down the aorta, the branches of the clot in the descending arteries often break loose from the aortic clot while those in the ascending aortic intercostal arteries are dragged or deflected into the aorta along with the principal clot. Especially low pressure areas are accordingly created in the chest walls at the cephalic ends of the pleural chambers. Such piezometric deflections can occur at numerous other points in the body.

In some cases of shock, as in some cases of peritonitis, the arterial looping ceases everywhere in the body except among some of the splanchnic vessels. Although oligemia develops and indicates loss of water from the blood to the tissues there is no ascites. In some cases of shock, as in some other cases of peritonitis, the looping of even the splanchnic arteries ceases completely or almost completely and water appears in the abdominal cavity. In other varieties of shock, when classified according to cause, the same situations arise.

In shock due to severe loss of blood the splanchnic arteries generally continue to loop and there is no ascites, but liquid does appear in the abdominal cavity in some cases when the heart beats for a considerable time so feebly that the splanchnic vessels can loop very little if at all. For a number of hours after the bleeding, water passes from the relatively high pressure areas in the intercapillary spaces into the blood stream until much of the lost blood is replenished, but in many cases this dehydration of the tissues is followed by a severe hydration of them. (Drinking excessive amounts of water undoubtedly increases the edema in some cases.) This edema is evidently due to an increased osmotic flow of water from the capillaries into the intercapillary spaces, for it sometimes develops while the heart action is very feeble. It is probably due to the circumstance that there has been very little or no looping of the arteries for so long that the tissues suffered severely in the absence of the normal mechanical exchange of respiratory gases and of nutrient and waste substances in general and that the osmotic pressure of the tissues accordingly exceeds that of the blood plus the tendency of the liquid in the intercapillary spaces to filter into the capillaries.

Schade and Menschel (1923) eliminated the mechanical hydration-dehydration differences by placing excised tissues in their own fluids. It was observed that the tissues became edematous. The same thing can be accomplished in a more usual way in the body by merely eliminating the systolic looping of arteries. In the absence of the arterial looping there is an absence of the mechanical hydration-dehydration difference that normally exists and that normally compensates for the greater osmotic flow of liquid from the blood into the tissues than from the tissues into the blood. The results of Schade and Menschel have been repeatedly verified in this laboratory and elsewhere and they are fundamental in the study of the exchange of body fluids.

SUMMARY AND CONCLUSIONS

1. The results indicate that the osmotic pressure of the tissue fluid of a mammal is normally greater than that of the blood.
2. Excessive hydration of the tissues does not occur, however, unless the tissue cells are especially injured, as by some chemical agent, or unless the

normal systolic looping of arteries either fails to occur or becomes greatly reduced.

3. The systolic looping of arteries gives rise to a mechanical dehydration of the tissues. This dehydration normally offsets the greater osmotic flow of liquid from the capillaries into the intercapillary spaces than the osmotic flow which occurs in the opposite direction.

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ABSORPTION OF WATER BY FATIGUED OR OTHERWISE IMPAIRED SKELETAL MUSCLE CELLS IN RELATION TO HEAT RIGOR

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In the summers of 1929 and 1930, the author ran a series of experiments, using the sartorius muscles from lots of thirty-five frogs in each experiment, from which Dr. Phillip Wells made careful determinations (among other things) of inorganic phosphorus and organic phosphorus present in the right sartorius muscles which had undergone long treatment, together, in isotonic alkaline (pH 9.8) or acid (pH 5.4) Ringer's solution, and check determinations on their mates—the left sartorius muscles—which had been carefully removed and dropped at once into liquid air.¹ The experiments were carried out by standard methods in use at that time. The results have never been published, but they confirmed the preliminary determinations made by Dr. Lawrence Irving and reported by Shafer (1928) in *THIS JOURNAL*. In the latter paper (p.155) the author pointed out, also, that although the treating solutions used were isotonic, the alkaline Ringer's solution gradually brought about, after long treatment, a slight increase in the weight and size of the muscles—i.e., the treating fluid caused changes within the living muscles such as to increase the osmotic pressure of the muscle protoplasm, with a resulting uptake of water. The acid Ringer's solution gradually caused a slight reduction in the weight and size of the muscles. It seemed reasonably clear that both acid and alkaline solutions brought about, within the muscle cells, a breakdown of large molecules into smaller molecules—evidenced by an increase in inorganic phosphates. These inorganic phosphates passed out of the muscle cells into the acid Ringer's solution, but, in the alkaline Ringer's, they were

¹ Two sets of experiments were run with acid Ringer's and two with alkaline Ringer's solutions. In one set, treatment was carried on until a barely visible response could be detected upon direct electrical stimulation of the muscles—i.e., 4 to 16 hours or more. In the other set, the treatment lasted 24 hours or more, until the muscles not only would not respond if stimulated, but were no longer capable of shortening during that rise in temperature (between about 35°C. and 45°C.) which ordinarily gave the first step in heat rigor of sartorius muscle of frog. The time when the latter condition was attained was determined by test muscles run in the same solution with the experiment muscles in each experiment.

kept within the cells—slowly accumulating, as the determinations showed, and raising the osmotic pressure.

The above studies were made in connection with the observed fact that muscles treated with the isotonic alkaline Ringer's solution (pH 9.8), for several hours, until they showed a barely recognizable response to electrical stimulation, then had a lower onset temperature of heat rigor; and the muscles similarly treated in the acid Ringer's mentioned showed a higher onset temperature of heat rigor than their normal mates. The increase of inorganic phosphates within the muscle cells was then looked upon as in some manner being the cause of the lowered rigor temperature—fatigued muscle probably having a lowered onset temperature of heat rigor for the same reason.

Vernon's experiments (1899) on the effect on heat contraction of varying the amount of water in muscle tissue—which he incorporated within the body of his longer paper on "Heat rigor in cold blooded animals"—had not yet come to my attention. In the summer of 1930, however, Vernon's experiments (1899), in which he showed that frog muscles treated with hypotonic saline solution entered heat shortening at a lower temperature and that muscles treated with hypertonic saline solutions required a higher temperature before heat shortening began, were repeated and confirmed. After that, lots of 35 left sartorius muscles of frogs were treated with either hypotonic NaCl (0.4 per cent) or hypertonic NaCl (1.5 per cent) solutions—the right sartorius muscles (mates) being dropped, untreated, into liquid air as checks for comparison—and Dr. Wells included these in his studies mentioned above. Here it was found that, within the limits of error of his method, the proportions of inorganic to organic phosphorus had not been modified by changes in the water content of the cells occasioned through treatments of the muscles with either hypo- or hypertonic NaCl solutions. The muscles were treated in the experiments with these solutions until one could barely detect a visible response upon giving a maximal electrical stimulation. This required from one and one-half to two hours of treatment. In that time, the muscles treated in the hypotonic NaCl solution gained an average of 0.43 gram per gram of muscle used; those in the hypertonic solution lost an average of 0.23 gram per gram of muscle used. In the case of other muscles given the identical treatment just described, those from the hypotonic solution entered rigor at a temperature about 10°C. lower than those from the hypertonic solution. The results indicated that it was the water imbibed because of the higher osmotic pressure occasioned by retained inorganic phosphates (with perhaps other salts also), and not the phosphates, as such, in the muscle cells, which had lowered the onset temperature of heat rigor in the case of muscles treated with isotonic alkaline Ringer's solution.

It was well known that fatigued muscles placed in Ringer's solution,

which was isotonic to non-fatigued muscle, imbibed water. Experiments were now carried out to learn whether muscles stimulated to fatigue within the body did so.

An apparently healthy, alert frog was selected and pithed. The sciatic nerve of the left leg was severed and the gastrocnemius muscle of that side (circulation intact) was stimulated through its nerve at five second intervals until it ceased to respond, followed by direct stimulation until it again ceased to respond (total period of stimulation lasting 30 to 35 minutes)—the muscles of the right leg remaining at rest. Then the fatigued and the non-fatigued gastrocnemii were removed carefully, cutting the tendons at corresponding positions, and the muscles were weighed. Four experiments were carried out in this way and in every case the fatigued muscle was heavier than its resting mate. The percentage increases in weight made by the fatigued muscles during the fatigue period (as determined by comparing with the weights of their resting mates) was as follows: 20 per cent, 15.7 per cent, 16.6 per cent, and 10.7 per cent—an average of 15.7 per cent increase in weight. This weight increase was due to water, as indicated by the fact that a dried, fatigued muscle weighed *very slightly* less than its unfatigued, dried mate.

Barcroft and Kato (1915), studying the effect of functional activity upon blood-flow and exudation in organs, found, after repeated stimulation of one gastrocnemius muscle of a dog, that this muscle weighed 19 per cent more than its resting mate—the circulation being intact during the stimulation period and for 8 hours and 15 minutes thereafter, before the muscles were removed and weighed. In the same laboratory Back, Cogan and Towers (1915) ran experiments, using frogs, in which one gastrocnemius muscle was stimulated with single induction shocks 40 times per minute for 15 minutes, while its mate was kept at rest—the circulation in both legs being intact. They found the stimulated muscles to be heavier than their mates. In one series (with brains of the frogs pithed but with the medulla and cord intact), they obtained an average gain in weight, in five experiments, of 4.3 per cent in the stimulated muscles over their resting mates. Since stimulation was carried on for only 15 minutes in their experiments, the muscles were evidently not completely fatigued; but the *active* muscles had become heavier and had a specific gravity slightly less than that of their resting mates.

Loeb (1894) first postulated that muscular activity should increase the molecules in solution in the muscle fiber and cause it to take up water with a pressure equal to the difference of the osmotic pressure built up in the fiber and that found in the blood.

In their discussion, Back, Cogan and Towers (above citation) remark, "It is clear that the phenomenon which we have observed can only take place when the muscle is well supplied with fluid from some external source."

The external source to which they refer would be the blood in their experiments, but they point out that in case of fatigued excised muscles the external source would be water with which the muscle is bathed.

In my own work, it had been determined that excised gastrocnemius muscles of the frog placed at once into a moist chamber (so that they could lose no water through evaporation) and completely fatigued, by repeated, direct, electrical stimulation, would then enter heat rigor at a little lower temperature than their unfatigued mates. Of course, muscles fatigued in moist air, in this way, did not gain in weight—they actually lost *very slightly* in weight, due, perhaps, to escape of carbon dioxide during the fatiguing process. If, now, it was really uptake of water by the muscle cells, which caused the lower onset temperature of heat rigor, then the cells in these muscles must have obtained their water from tissue fluid within their own tissue spaces. That is, the cells must have taken up *some* water and increased in size somewhat at the expense of their tissue fluid. In order to prove whether it was the muscle cells, themselves, which took up water during a prolonged series of contractions the following plan was used to measure skeletal muscle cells of frog's sartorius muscle before and after the fatiguing process.

In the fall of 1930, very small young frogs of the species *Rana boylei* were obtained from water holes in the foothills near Stanford University. From the smallest of these an extremely thin sartorius muscle could be dissected out. When such a muscle was carefully dissected, it was found that the first two or three muscle cells along the border edge could be distinguished as *individual* cells whose width could be rather sharply made out under the compound microscope. In order that the microscopic width of these individual muscle cells might be measured both before and after being fatigued, under the same moisture conditions, a very cheap but satisfactory moist cell containing electrodes was constructed on an ordinary microscope slide. First, a 1 mm. thick glass plate was smoothly cut to 6 mm. wide by 30 mm. long. Two narrow, thin tinfoil electrodes were sealed down over magnet wires on a clean microscope slide—by means of tough wax—so that the free ends of the electrodes were about 15 mm. apart in the middle of the slide. Over these free ends of the electrodes, then, was laid the 30 mm. glass plate, with its length parallel to the length of the slide, and equally spaced over the electrodes. While the glass plate was held down tightly, melted paraffine was poured to surround and cover the plate and the exposed ends of the electrodes on the microscope slide. When the paraffine had cooled, a safety razor blade was used to shear all paraffine away down to the level of the surface of the glass plate—the plate itself serving as a guide to the razor. Thereafter, the plate was lifted out carefully, leaving a well in the paraffine of the size of the plate and 1 mm. deep. At the bottom of this well, a 7 mm. length of each electrode was

left freely exposed. These exposed tinfoil electrodes were now made to cup upward to the extent of 1 mm. so that they could act as very delicate spring electrodes. They could be easily depressed with very slight pressure and if the pressure were lessened, they would again cup upward. A thin, perfectly flat cover glass of suitable size for the paraffine well was provided. The muscle was dissected; fine silk ligatures were tied to its tendons, and it was lifted from the frog's leg and laid flat, with its ends resting upon the cupped electrodes, in the shallow paraffine well. The ligatures were tied under the magnet wires at the ends of the slide and then drawn tightly until the muscle was placed slightly but distinctly on the stretch, when each ligature was made secure so that the muscle could not shorten. Where the fine ligatures lay taut across the paraffine walls at the ends of the cell, they were lightly touched now with a hot needle until they were sealed just flush with the surface of the paraffine. The sheared surface of the paraffine walls was made wet with a little Ringer's solution (and if the upper surface of the muscle was not still entirely wet with tissue fluid, it was touched with Ringer's solution). Ringer's solution was used to wet the under surface of the cover glass and the latter was at once brought into place over the well. Surface tension drew the cover glass into wet contact, all around, with the evenly sheared paraffine walls and with the flat upper surface of the muscle. Under proper manipulation, capillarity drew the thin free border of the muscle out into close contact with the under side of the glass cover. Thus the shallow paraffine well became a perfect moist chamber in which the muscle was held by its ligatures so that it absolutely could not shorten. Otherwise, it was free, except that it was in contact only gently with the cover glass from above; and from beneath it was touched lightly at the ends by the cupped tinfoil electrodes. Under the microscope, reflected light could be thrown through the very thin border of the muscle so that two or three muscle cells, nearest the edge, could be focussed on distinctly with a Leitz 7 objective and I eyepiece. The cross striations could be seen and the width of the cells chosen could be measured. The first measurements were made immediately after the adjustments described above were completed—with the muscle at rest. Then quick, repeated, maximal, electrical stimulations were begun and continued for 20 to 30 minutes, until the muscle ceased to make a visible response. At each stimulation of the fresh muscle, the cross striations of the cells in the field would be seen to make a *very slight*, quick vibration back and forth under the microscope. After the muscle had been fatigued, the widths of the same individual cells were measured again. The line of division between cells was often more difficult to make out after fatigue; but when one had made himself acquainted with certain landmarks that could always be discovered along any particular cells under observation, the division boundaries could then be distinguished. The cells were found, consistently,

to measure slightly greater in width after fatigue than before. Since the cells could not become shorter, this increase in width (diameter) could only mean that the volume had increased.

The young frogs were collected and the first results were checked during the fall of two succeeding years. Measurements in the first series of experiments were carried out with an eyepiece micrometer. In the second series, photomicrographs were made, first, of the unfatigued cells, and afterward of the same cells again (without moving the microscope or the camera) when the fatiguing process was over. Measurements of the width of the cell photographs (before and after) were obtained to the thousandth of an inch with a steel micrometer. In this series sufficient tissue fluid was used to cover entirely the muscle in each case without the use of Ringer's solution. The third series of measurements was made with a direct illumination objective magnifying 230 times, using an eyepiece micrometer, as in the first series. For comparison, all the results were computed as percentage increases in width of the cells. Results from the most accurately defined cells of the first series were: 16 $\frac{2}{3}$; 11; 11; 20; 4; 18 and 20 per cent—an average of 14.3 per cent increase in the diameter of the cells. Results from the second series (the photographed cells) were: 16; 20; 20; 16.6; 12.7; 10.4 and 13 per cent—an average of 15.5 per cent increase in diameter. Actual measurements of the width of the photographs of one of these cells taken before and after fatigue is given here:

	<i>inch</i>
2nd cell from margin, after fatigue.....	0.291
2nd cell from margin, before fatigue.....	0.258
Gain in width.....	0.033

$$\text{Percentage increase} = \frac{0.033 \times 100}{0.258} = 12.7 \text{ per cent}$$

The third series gave the following results: 20; 18; 11; 7; 20; and 15 per cent—an average increase of 15 per cent in diameter. The second series, in which the cells were photographed and their photographs measured, appears to be the best—since it gave the most uniform results. The average of the averages of the three series gives 14.9 per cent as the percentage increase in width or diameter made by the cells. Since the volume of the more or less cylindrical muscle cells would vary with the square of the diameter, the percentage increase in volume of these cells, as indicated by the above average increase in width, would be somewhat greater than the average percentage increase in weight found for completely fatigued muscles as compared with their unfatigued mates when the trial was made in the frog's body with the circulation intact. After fatiguing one of the small stretched muscles, in its moist chamber, it would sometimes lift the cover glass slightly, which may indicate that, as the cells took up water

during the fatiguing process, they tended to press against the cover (which was held to the paraffine wall by capillarity) with a little greater pressure than before fatigue. This would tend to flatten the cells against the cover slightly, and might cause the visible width to measure slightly greater than it should. That the cells do become actually wider, however, there can be no doubt; and since, in the second series, each muscle was bathed only with a plenteous supply of tissue fluid, we have proof that individual skeletal muscle cells take up water from tissue fluid (or from Ringer's if it is used) and become larger during repeated contractions—and that this must regularly take place in rapidly contracting, fatiguing muscles in the living body. It is the extra water taken up by the fatigued or partly fatigued cell which, in some manner, causes the cell to enter heat rigor at a lower temperature than does the unfatigued, well oxygenated or recovered muscle cell. Moreover, whatever method is used to cause the muscle cell to imbibe water appears, thereby, to lower the onset temperature for heat rigor in that muscle. It has already been pointed out that surrounding a muscle with a hypotonic solution would cause the rapid uptake of water, by the cells, without altering materially the proportion of inorganic to organic phosphorus in the muscle, but, nevertheless, with the lowering of the onset temperature of heat shortening. Still another method of lowering heat rigor temperature in muscle may be given and shown to depend upon uptake of water.

Lippay and David (1932) and Martini (1932) have already published that the frog's gastrocnemius muscle, poisoned with monoiodoacetic acid has a lowered onset temperature for heat rigor. The author has experiments which confirm their results, but show, in addition, that a poisoned gastrocnemius muscle in a frog poisoned with monoiodoacetic acid becomes heavier, from imbibed water, than its unpoisoned mate in the opposite leg which has been tied off previous to administering the poison. A single experiment may be related. A frog was pithed without loss of blood. The right leg was ligatured just above the knee and the part below the ligature removed. Its gastrocnemius muscle was dissected at once and weighed (0.46—gram). The frog was then injected in the dorsal lymph sac with 0.4 m. gram of monoiodoacetic acid per gram of intact frog. The sciatic nerve of the left leg was isolated at once without loss of blood and cut. Forty-five minutes later the muscles of the frog, except those below the cut sciatic of the left leg were becoming stiff with rigor. The left gastrocnemius was removed quickly and found to weigh 0.49 gram. It was brought into the same moist chamber with its unpoisoned mate—the two being attached to equal, separate levers. As heat was applied the poisoned muscle was already entering rigor; shortening slowly at 26°C., and more rapidly at 30°C. The unpoisoned mate began heat rigor shortening at 36°C. The poisoned muscle had made a 6.5 per cent increase in weight:

the explanation of its behavior would seem to be as follows. The muscle poisoned with moniodoacetic acid is thereby deprived of its lactic acid metabolism (Mawson, 1932) and unable, because of that, to carry on efficiently the necessary restorative oxidative processes. As a result, waste products of catabolism in the cells raise their osmotic pressure so that they take up water from the tissue fluids. Coördinated with this uptake of water by the living muscle cells is the lowered temperature at which the muscle goes into heat rigor. Of course, at ordinary room temperature the excised muscle whose cells have taken up extra water goes into rigor mortis more rapidly than does the excised normal muscle.

This phenomenon of the uptake of water by skeletal muscle cells under conditions where the cells have incurred an oxygen debt is of wider significance. For example, an increase in the specific gravity of the blood is said to take place in strenuous exercise. Jones (1887 and 1891) first reported that mild exercise causes a slight lowering in specific gravity, and that violent exercise (or exercise with perspiration) quickly brings about a sharp rise in the specific gravity of the blood. By inference this author looked upon the increase in perspiration as the explanation of the rise in specific gravity. Barcroft and Florey (1929), and Barcroft and Poole (1927) have shown that the spleen, contracting strongly during heavy exercise, plays a rôle in the increment of red blood corpuscles in the blood during exercise—as also during asphyxia.

This being true, the spleen may thereby play some part in increasing the specific gravity of the blood in the general circulation during exercise. Certain waste products, such as lactates, escaping from active cells undergoing rapid catabolic changes, would tend, also, to raise the specific gravity of the blood.

However, when one considers that the voluntary muscles of an athletic man constitute about half the entire weight of the body, it is easy to see that should this great bulk of muscle tissue increase its weight by even 5 per cent² through uptake of water in strenuous exercise, the volume of water required would amount to quite one-half of the normal volume of the blood. That such an actual fall in water content of the blood would take place under the circumstances is unbelievable—and would not necessarily have to occur. No doubt, as water began to be lost to the muscles, the blood itself would begin to draw water from inactive tissues of the body, such as the liver and the subcutaneous tissues. We do not know how nearly toward complete fatigue all the muscles may be voluntarily driven (a 5 per cent increase in weight for the entire musculature may be too low, or too high, an estimate) although, in a strenuous rowing or running race where all the muscles are in heightened activity the oxygen debt incurred

² I.e., instead of 15 per cent or so, as has been proven to be the case (on the average) when a single gastrocnemius muscle of the frog is completely fatigued by direct stimulation in the body with the circulation intact.

is proven to be very great. Therefore, in any case, it seems clear that this established phenomenon of uptake of water, by severely fatigued muscle cells, would be an extremely important factor—perhaps the most important factor—in causing the reported increase in specific gravity of the blood during strenuous exercise. The active muscle cells would take up water from their own tissue fluid, the latter would be replenished from the blood and the water taken from the blood would be restored to it *in part only* from the inactive tissues.

Further significance is seen in the plain implication that the phenomenon in question, probably, is not specific to strenuously active muscle alone, but also holds true for other active tissues—e.g., glands and nerve tissue. Indeed, one may point, here, to the direct evidence which would be afforded by the work of Dolley (1909), and others, in case it should become fully confirmed. Dolley concluded, from histological studies, that as a result of continued activity, changes occur in the Nissl substance and in the intranuclear substance of the Purkinje cells of the dog's cerebellum, which are attended by an increase in the size of the cell bodies named and in the size of their nuclei. This increase in size he describes as an edema. Kocher (1916) denies this finding. He says that any differences in cell size found by histological methods are within the limits of normal variation found regularly in case of the neurons in question.

It should be added that Dolley's photomicrographs (all made to the same magnification) of the resting neuron and of neurons taken after different stages of fatigue, show increases in the size of the Purkinje neurons as the granular substances within the protoplasm break down in successive stages of fatigue.

More recently, Bast and Bloemendal (1927) in the fourth of a series of papers, from Bast's laboratory, on "Studies in experimental exhaustion due to lack of sleep," associate chromatolysis and appearance of vacuoles in the cytoplasm with certain neurons in exhausted rabbits. They do not state whether the appearance of the vacuoles was associated with any change in cell size or not. Their figures 6, 7, 8 and 9 show neurons, from the reticular formation of the medulla of exhausted rabbits, as decidedly larger than the cell in figure 5 from the same formation in a normal rabbit. Presumably these figures represent the same magnification although that is not stated. In the third paper of the series, Bast, Schaecht and Vanderkamp (1927) state that they "observed no shrinkage of the nucleus or cell as many workers report." They do not state, however, whether they observed any increases in neuron size to accompany exhaustion. The difficulty of coming to a certain conclusion in that matter, by histological methods, is apparent when they state that variations in size *do* occur in neurons of a particular region in the normal healthy animal. The question as to whether extreme fatigue causes a neuron to take up water from tissue fluid and become larger, therefore, cannot be regarded as settled.

The uptake of extra water by functioning neurons, or any portions of neurons, or possibly by any intermediary substance between a functioning neuron and the tissue it activates, if it should be shown to *actually take place*, would assume evident aspects of importance in connection with the question of impairment of irritability and conduction. The known facts with respect to fatigue and heat rigor in muscle lead one to ask the question: Does heat rigor (death) of fatigued neurons begin at a lower temperature than in normal non-fatigued neurons?

SUMMARY

1. Fatigued skeletal muscle cells (intact in the living body, or excised) and muscle cells treated with isotonic alkaline Ringer's solution (pH 9.8) until they barely make a visible response to electrical stimulation, have an increased osmotic pressure. As a result, they regularly take up water from their tissue fluids or from ordinary Ringer's fluid with which they may be bathed, and become larger and heavier.

2. Proof that it is the muscle cell, itself, which increases in size (through uptake of water from tissue fluid) as a result of fatigue, is afforded by measurements of individual muscle cells made both before and after fatigue.

3. The muscle cell which has taken up water as a result of fatigue, or because of treatment with alkaline Ringer's mentioned in no. 1 above, or as a result of being bathed with a hypotonic salt solution, or through poisoning with monoiodoacetic acid, goes into heat rigor at a lower temperature than does normal, non-fatigued muscle. It is the extra water which the protoplasm has imbibed that somehow causes the lowered onset temperature of heat rigor.

4. The significance of the uptake of water by strenuously active muscle cells is discussed, and the question as to whether this phenomenon is true for other active tissue cells—especially neurons—is raised and discussed.

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THE REVERSIBLE HEAT SHORTENING OF ELASTIC CONNECTIVE TISSUE, AND AN EXAMINATION OF THE POSSIBLE EFFECTS OF CERTAIN FACTORS ON THE HEAT SHORTENING OF CONNECTIVE TISSUES

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In a paper on the effect of fatigue on heat rigor and chloroform rigor in the gastrocnemius and sartorius muscles of the frog, the author (Shafer, 1928) drew attention to the fact that only the first step in heat rigor shortening was to be associated in a causal way with the shortening of muscle in normal contraction—namely, the step in heat rigor shortening which is directly affected by previous fatigue. Attention was also called to the shrinkage or shortening of tendons (white connective tissue fibres) at temperatures above 58°C. and the relation of such shortening to the second and third steps of heat rigor described by Brodie and Richardson (1899). The tendon tissue shortening may begin as low as 48° to 50°C. and definitely include both the second and third steps mentioned. No consideration was given to the possible effects of heat upon yellow, elastic connective tissue fibers in the muscles studied, of which an extremely small amount appears to be present—i.e., in the walls of those arterioles which are between the fascicles of the muscles, if not also in the limited areolar tissue of the frog muscle.

The present paper gives a brief statement of results obtained from a series of experiments made to determine:

1. Whether elastic connective tissue (as well as white connective tissue) shows a "heat-shortening" upon sufficient rise in temperature.
2. Whether changes in pH (within the physiological limits of pH 5.4 and pH 9.8), or changes in osmotic pressure of a salt solution with which the excised connective tissues may be bathed, affect the onset temperature of heat-shortening in the tissues.
3. Whether any onset temperature of heat-shortening found in connective tissues under the conditions studied could affect (or help determine) the onset temperature of heat rigor of muscle—especially that of frog muscle.

The onset temperature of shortening of yellow, elastic connective tissue was studied in the aorta of the bull frog in order to obtain as large pieces

of nearly pure elastic tissue of the frog as possible. For comparison, it was also studied in the aorta of the ox; and a few trials were run with pieces of yellow connective tissue from the ligamentum nuchae of the ox and with pieces of aorta from the white rat. Forty-five experiments were carried out. In most cases—i.e., whenever a comparison was to be made—two

TABLE I
Normal tissues taken directly from the body and heated in the same moist chamber in pairs
Temperatures are given in Centigrade scale

EXPERIMENT NUMBER	TISSUE USED	ANIMAL TAKEN FROM	ONSET TEMPERATURE OF SHORTENING OF WHITE CONNECTIVE TISSUE	APPARENT ONSET TEMPERATURE OF SHORTENING OF ELASTIC TISSUE	TEMPERATURE AT WHICH ELASTIC TISSUE BEGAN TO SHORTEN MORE RAPIDLY, AND REMARKS
			degrees	degrees	
14	White tendon Aorta	Bull-frog Bull-frog	54.5	26.5	Distinctly so at 56°
15	White tendon Aorta	Young bull-frog Young bull-frog	52	26	53°
16	Smooth muscle with admixture of white connective tissue	Cross cut of stomach of bull-frog	58		Lengthened slowly from 28° to 58° where rapid shortening began
	Aorta	Bull-frog		28	56.5°
18	White tendon Aorta	Large bull-frog Large bull-frog	58.5	24	58.5°
1	White tendon Yellow Lig. nuchae	Sheep Ox	61.5	29	62°
5	White tendon Aorta	White rat White rat	60	28	60°
7	White tendon Aorta	Calf Calf	58	28	Not distinctly until 61°

pieces of tissue, to be compared as to their onset temperatures of heat-shortening, were confined in the same moist chamber under the same conditions of temperature change. They were attached to two exactly similar levers arranged to record on a kymograph outside of the chamber, one lever writing behind the other, from the same base line. The ratio of the

lever arms used was 216 mm. for the writing arm to 33 mm. for the arm to which the tissue was attached.

Table 1 gives, as typical, a selection of seven of these comparisons of normal tissues of different animals.

Table 2 gives eight typical comparisons of treated tissue with exactly similar normal tissue from the same animal.

Most of the experiments in the tables were carried out in a room temperature ranging from 20° to 22°C.—one, as mentioned later, at a higher temperature. Very soon after the temperature of the tissue chamber began to rise, the elastic tissues began to show a slow and gradual heat-shortening indicated in the table as the *apparent* onset temperature of shortening. This gradual shortening was continuous, as the temperature increased, until a temperature was reached a little below that at which a second more rapid shortening began—the latter occurring at a temperature which was always within the range at which white connective tissue starts to shorten. Regularly there was a plateau, or even a noticeable decline (fig. 1, from *d* to the 63° point) in the first gradual curve of shortening just before the second or more rapid shortening began. The similarity in the onset temperature of this more rapid second shortening in pieces of aorta (or of elastic ligaments) and the onset temperature of heat shortening in white tendons is shown in table 1. As the table shows, also, white connective tissue (tendons) of the frog usually began shortening at a lower temperature than did pure white connective tissue in tendons of mammals. This point is well known. However, some wide variations were met with in the onset temperature of heat-shortening in white tendons of frogs—ranging from 48° to 62°C. The range, in this respect, for white tendons of the mammals studied was from 58° to 62°C. inclusive—thus the upper and the lower limits of the range in frogs and in mammals, respectively, overlapped.

Table 2 shows that no significant variations in the onset temperatures of shortening of the connective tissues were caused either by the acid or the alkaline Ringer's solutions used, or by the hypotonic and hypertonic salt solutions with which the tissues were treated. Some wider variations than are given in this table were found but they were entirely within the limits of the range for normal tissues.

A part of the author's heat rigor work with frog muscle (i.e.) was carried out at a lower room temperature (about 19°C.) than were these with connective tissue given in table 1. It was deemed advisable, therefore, to carry out experiments with elastic tissue starting at a temperature several degrees lower as well as somewhat higher than 22°C. Experiment 43, in table 2 for example, was carried out starting with a tissue temperature of 25°C. It will be seen that the apparent onset temperature for heat shortening of the aorta, in that experiment, was higher than in other experiments recorded. Ice was used to bring the tissue chamber and its contents to

42	Right aorta	Bull-frog	In Ringer's at pH 5.4, 18 hours Normal	See remarks	26	Began to shorten rapidly at 53°
	Left aorta	Bull-frog				Began to shorten rapidly at 56°
43	Right aorta	Bull-frog	In Ringer's at pH 9.6 with Ca(OH)_2 , 18 hours Normal	See remarks	30	Began to shorten rapidly at 54°
	Left aorta	Bull-frog				Began to shorten rapidly at 56°

17°C. When this was done, and then the temperature of the chamber was gradually raised, the apparent onset temperature of heat shortening in pieces of aorta of the cow was found to be 20° to 22°C. After the temperature had been raised (with slow continuous shortening of the piece of aorta) up to 40° or 47°C., then if the chamber were cooled again, the tissue lengthened to its original state—and the process could be repeated. Thus, it soon became apparent that excised elastic connective tissue regularly changed length, becoming shorter with higher temperatures and longer with lower temperatures—i.e., for temperatures between 17°C. and the onset temperature for the second rapid shortening. Later, in one experiment, the temperature was brought to 8°C. and the tissue continued to lengthen slowly during the change down to that temperature. White connective tissue, on the other hand, did not change length until a certain temperature range was reached. White tendon of the cow, for example, did not change length until about 61° to 62°C. (typically) was reached, when it started to shorten comparatively rapidly with further increase in temperature; then, upon cooling, it did not regain its original length. When the temperature of a piece of cow aorta was raised until it began its second more rapid shortening, it continued to shorten to 90° or 94°C. At this temperature it was still elastic, and, when cooled, the tissue lengthened again. However, most of the shortening which took place in that part of the temperature range which was above the onset temperature of shortening of white connective tissue was a *permanent* shortening. This may be taken as a further indication that the second and steeper portion of the heat-shortening curve of pieces of aorta (or of elastic ligament) is really due to a small amount of white connective tissue present which has superimposed its effect upon the more gradual curve of the elastic tissue. Of course the same effect could conceivably be produced by some constituent (or by some structure), in the elastic tissue itself, similar to that in white connective tissue. However, the effect is most likely due to white fibers, since some white collagen fibers are known to be present in all yellow elastic tissue.

Figure 1 is a graphic record of an experiment with a piece (about 35 mm. long and 8 mm. wide) of aorta of cow attached so that shortening caused the lever to rise, and lengthening of the piece, as it cooled, cause the lever to decline or fall. The base line was written by the attached lever at 22°C. Beginning at *a* the temperature was raised to 50°C.—the drum remaining stationary. Then the drum was moved by hand from *a'*, which it had reached at 50°C., to *b*. The tissue chamber was cooled by ice to 17°C.—the lever slowly falling with the temperature to *b'*. The drum was moved by hand to *c*, and the temperature was gradually raised to 50°C. again, by which time the writing point of the lever stood at *d*. (Practically, the lever had ceased to rise at about 47°C.) At 50°C. the drum was started from *d*,

slowly, as the temperature continued to rise. The usual plateau, already mentioned, is seen—in this case with a slight decline; this decline is not always observed in similar experiments. Then, at 63°C. the lever began the second, the more rapid, rise. The drum was stopped again at d' at 90°C., and the temperature continued to increase to 93.5°C. After that, the tissue chamber was allowed to cool slowly, over a period of 30 minutes, until the lever came to e at 60°C. At e ice was used to lower the temperature more rapidly down to 17°C., when the drum was moved to f by hand; and the lever was raised again as the temperature was gradually brought up to 94°C.

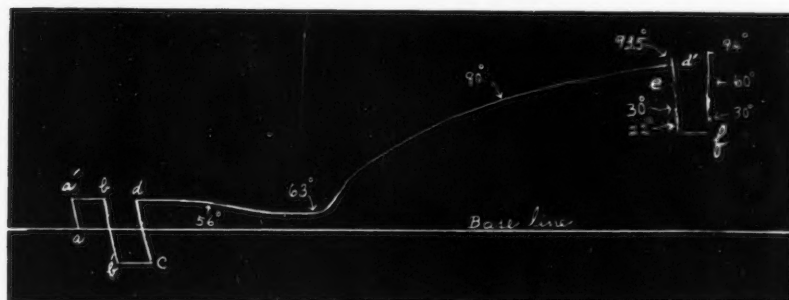


Fig. 1. Graphic record written by a strip of aorta of a cow, showing reversible and non-reversible changes in length with changes in temperature. Full description in the text.

The cause of the plateau, and of the decline in the plateau, would seem to be due to the very small amount of smooth muscle fibers known to be present in the wall of the aorta. Excised smooth muscle lengthens very gradually, and at a slightly increasing rate, beginning at about 28°C. and continuing up to nearly the temperature at which white connective tissue begins heat-shortening. If this lengthening of the smooth muscle fibres should become relatively more rapid than the rate of shortening due to the elastic tissue in the plateau portion of the curve, it could thus cause the slight decline as seen in figure 1, beginning at about 56°C. Note that the lever fell from its position at 93.5°C.—and still further from its position at e (60°C.)—as the tissue chamber was cooled, and that it was lifted again at f , when the temperature was increased, just as it did at b and at c , respectively, before the tissue had been heated to 93.5°C.; the range of the lever, however, being at a *new, higher level* with respect to the base line. This shows that the piece of aorta had become permanently shorter by nearly the amount of the second, or more rapid, shortening which occurred between 63° and 93.5°C.—viz., the shortening which occurred during that rise of

temperature in which white connective tissue is known to become permanently heat-shortened. Moreover, the rise at f from 17° to 94°C. was a *continuous* rise. Obviously, this behavior was obtained at f because the effect of both the white connective tissue and the smooth muscle had been destroyed by the first heating up to 93.5°C. ; and the behavior thereafter (i.e., at f) was due to elastic tissue alone. The elastic tissue in these experiments was not submitted to long continued high temperature.

It seems not unreasonable to suppose, from these results with excised tissues, that elastic connective tissue fibers may change length with changes in temperature in the living body; and that the *large* arteries may, therefore, become slightly smaller but capable of a little more elastic stretch at fever-temperatures than at subnormal temperatures under corresponding conditions of blood pressure.

Attention should be called to the peculiar behavior of pieces of white connective tissue (tendon of ox) treated with hypertonic salt solution. This tissue increased in weight by a rather definite amount in hypertonic NaCl solution as well as in hypotonic solutions—an average increase of about 0.12 gram per gram of tendon used. Even in an 8 per cent solution of NaCl, pieces of white tendon made their decided gain in weight. However, after as much as 71 hours treatment (in the ice box), in 8 per cent NaCl solution for example, a piece of tendon of bull began its heat-shortening at 62°C. Likewise a similar piece of white connective tissue tendon, treated 66 hours in hypotonic (0.2 per cent) NaCl, began heat-shortening at 62.5°C. There had been no visible change in the appearance of the treated pieces, and the onset temperature of heat shortening was not different from that of untreated tendon; but these two pieces of tendon, which had been submitted to a very long soaking (the one in 8 per cent and the other in 0.2 per cent NaCl solution) underwent the most extreme heat shortening observed in connective tissue, as the temperature increased between 62° and 90°C. —viz., they shortened to between one-third and one-fourth of their original length, and they increased in thickness to about twice their diameter before heat was applied.

From the series of experiments, as outlined above, it became clear that the well known, extensive, rapid, permanent shortening which takes place during the first step in heat rigor of skeletal muscle could scarcely be confused with the first, very slow, gradual, reversible changes in length caused by changes in temperature of elastic tissue. Neither could confusion arise in connection with the second, more rapid, permanent phase of heat shortening observed in pieces of elastic connective tissue. The latter phase of shortening corresponded in temperature range with the second step (or second and third steps, since these are often indistinguishable), in heat rigor of muscle—a shortening which has been shown to be due to white connective tissue in the muscle, and which is almost certainly due, in

the case of elastic tissue, to the small admixture of white fibers always present in that tissue. Moreover, none of the treatments of freshly excised elastic or inelastic connective tissue with the hypertonic or hypotonic salt solutions named, and no treatment with Ringer's solutions at pH values between 5.4 and 9.8, served to modify either kind of connective tissue so that its heat-shortening could be confused in any way with the shortening exhibited during the first step of heat rigor of muscle—i.e., the true heat rigor of the muscle cells.

SUMMARY

1. Excised elastic connective tissue shortened with increases in temperature and lengthened again with decreases in temperature within the range from 47° down to 8°C., which was the lowest temperature at which the tissue was tested. This behavior of fresh, excised, elastic connective tissue indicates that the larger arteries, in the living body, must tend to become *very slightly* smaller (and correspondingly more stretchable) at fever temperature than at subnormal temperatures under similar conditions of blood pressure.

2. The relation of the small amount of smooth muscle and of white collagen fibers, present in the pieces of elastic tissue studied, to changes in length during change in temperature is considered. Pure elastic connective tissue (effect of smooth muscle and collagen fibers destroyed by heat) exhibited reversible changes in length with changes in temperature from 8° to 94°C. Higher or lower temperatures were not tried.

3. White connective tissue does not change length with change in temperature until an onset temperature for permanent heat-shortening is reached, which is typically about 62°C. in the case of tendons from mammals and about 48°C. for tendons from frogs. The range of this onset temperature is discussed.

4. Treatment with decidedly hypertonic and hypotonic salt solutions (8 per cent to 0.2 per cent NaCl), and treatment with Ringer's solution at pH values between 5.4 and 9.8 did not modify either kind of connective tissue so that its heat-shortening could be confused in any way with the shortening exhibited by muscle tissue during its first step of heat rigor—viz., the true heat rigor of muscle cells.

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LACTIC ACID IN BLOOD AND TISSUES FOLLOWING INTRAVENOUS INJECTION OF SODIUM BICARBONATE

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Some years ago Macleod and Hoover (1918) reported an increase in blood lactic acid following intravenous injection of sodium carbonate. Similar results with bicarbonate injection were recently obtained in artificial respiration experiments by Gesell, Krueger, Gorham and Bernthal (1930). The object of the present investigation was to ascertain the comparative effects of bicarbonate injections on the lactic acid of the blood and tissues with pulmonary ventilation under normal physiological control.

METHOD. A molar solution of sodium bicarbonate was injected either into the femoral or jugular vein of small dogs (3 to 7 kilos) anesthetized with morphine and urethane. In eleven experiments 20 cc. per kilo body weight were injected; in four experiments 18.5 to 29.3 cc. Injection was fairly rapid at approximately 20 to 30 cc. per minute. Respiration and blood pressure were followed on a kymograph record. Room air was breathed from a rebreathing tank. Fifteen to thirty minutes after injection the animals were decapitated with a T-shaped guillotine in the manner described in a previous paper (Haldi, 1932). The brain and kidney were removed immediately after decapitation and a portion of the brain and one kidney dropped into liquid air within a few seconds. The exact time, recorded with a stop watch, is given in table 2. A corresponding piece of brain tissue from the same animal and the other kidney were incubated at 38°C. for either five or ten minutes before freezing. It was thought that bicarbonate might possibly have the specific effect of accelerating lactic acid production in the tissues, and that if such were the case, it might become manifest in a more rapid accumulation of lactic acid in the incubated brain and kidney after injection. As a basis of comparison a series of control lactic acid values were obtained from the brain and kidney of a number of animals anesthetized with morphine and urethane but without injection of bicarbonate.

The effects of bicarbonate on muscle lactic acid were studied by comparing the lactic acid content of the sartorii, one excised before, the other after injection. The muscles were carefully exposed and freed from adjacent tissue without injury to the blood vessels or nerve. They were then removed by cutting with a scissors near the points of origin and insertion

and dropped at once into liquid air. Bleeding in the animal was prevented by clamping on a hemostat when the muscle was excised.

Blood samples were taken from the femoral artery before and after injection. The last blood sample was withdrawn two to four minutes before the animal was guillotined. In several experiments urine collected shortly before the injection and again following the second blood sample after injection, was analyzed for lactic acid. The bladder was emptied

TABLE I
Control series—animals anesthetized with morphine and urethane

EXPERIMENT	TIME INTERVAL BETWEEN DECAPITATION AND IMMERSION OF BRAIN IN LIQUID AIR	LACTIC ACID IN BRAIN	INCREASE IN LACTIC ACID OF BRAIN INCUBATED 5 MINUTES	INCREASE IN LACTIC ACID OF BRAIN INCUBATED 10 MINUTES	TIME INTERVAL BETWEEN DECAPITATION AND IMMERSION OF KIDNEY IN LIQUID AIR	LACTIC ACID IN KIDNEY	INCREASE IN LACTIC ACID OF KIDNEY INCUBATED 5 MINUTES	INCREASE IN LACTIC ACID OF KIDNEY INCUBATED 10 MINUTES
	<i>seconds</i>				<i>seconds</i>			
1	9	49.4*	84.7		14	25.2	12.1	
2	8	51.1		136.6	12	35.1		31.9
3	10	56.0		99.4	14	22.3		29.8
4	10	60.2	102.0		23	22.8	20.1	
5	6	42.4	88.7		22	20.2	39.8	
6	37	86.1		108.3	14	26.0	34.5	
7	19	66.9		121.5	34	35.7		39.3
8	15	68.5		103.3	40	44.8		25.3
9	44	84.5		102.0	40	37.4		49.1
10	47	76.1		145.5	36	37.1		40.0
11	20	65.2	98.0		16	20.9	30.5	
12	17	57.9	117.5		26	28.3	26.8	
Average experiments 1-5.....	8	51.8						
Average, all experiments.....	20	63.7	98.2	116.1	24	29.8	27.3	35.9

* Lactic acid in tissues is expressed throughout in terms of milligrams per cent.

by pressure of the hand inserted through a slit in the abdomen. Substances interfering with lactic acid determinations in urine, blood and tissues were removed by the copper-lime treatment described by Peters and Van Slyke (1932). Lactic acid analyses were made by the Friedemann, Cotonio and Schaffer method (1927). Checks were obtained by running triplicate determinations on each sample.

RESULTS. *Brain.* Inspection of tables 1 and 2 reveals that in a number of experiments a relatively long interval elapsed between decapitation and

TABLE 2
Injected animals—anesthetized with morphine and urethane and injected with sodium bicarbonate

EXPERIMENT	MOLAR NaHCO_3 INJECTED PER KILO BODY WEIGHT	TIME INTERVAL BETWEEN DECAPITATION AND IM- MERSION OF BRAIN IN LIQUID AIR	seconds	LACTIC ACID IN BRAIN	INCREASE IN LACTIC ACID OF BRAIN INCUBATED 3 MINUTES	INCREASE IN LACTIC ACID OF BRAIN INCUBATED 10 MINUTES	TIME INTERVAL BETWEEN DECAPITATION AND IM- MERSION OF KIDNEY IN LIQUID AIR	seconds	LACTIC ACID IN KIDNEY	INCREASE IN LACTIC ACID OF KIDNEY INCUBATED 5 MINUTES	INCREASE IN LACTIC ACID OF KIDNEY INCUBATED 10 MINUTES	LACTIC ACID IN MUSCLE BE- FORE INJECTION	LACTIC ACID IN MUSCLE AF- TER INJECTION	INCREASE	LACTIC ACID IN BLOOD BE- FORE INJECTION	LACTIC ACID IN BLOOD AF- TER INJECTION	INCREASE
13	20.0	9	49.3*	105.3			21	30.5	18.0			49.9	59.0		11.0	30.4	19.4
14	20.0	12	61.3	94.1			24	41.4	14.3							55.0	38.2
15	20.0	5	52.7	92.2			20	35.9	32.4							59.2	26.7
16	20.0	5	55.5	102.3			14	51.9	22.8			61.3	67.2			54.6	38.9
17	20.0	7	52.9	127.0			20	63.3	25.1			50.3	68.8			61.9	38.3
18	20.0										18.4	64.4	78.8			43.0	24.1
19	22.7	21	58.6		130.2		18	44.1								42.5	16.3
20	29.3	39	81.5		129.0											50.5	24.3
21	18.5	22	76.2		162.8		22	46.5			25.8					50.9	29.9
22	20.0	32	76.8		114.6		32	69.4			32.0					47.7	14.1
23	22.7	28	96.6		114.6		26	43.8			40.2					48.0	36.6
24	20.0						15	28.1			33.3					50.2**	
25	20.0											62.8	71.3	8.5		64.5**	
26	20.0											52.9	68.7	15.8		50.4**	
27	20.0											65.9	66.0	0			
Average experiments 13-17.....	8		54.3														
Average all experi- ments.....	18		66.1	104.2	130.3		21	45.5	22.5	29.9		58.2	68.5	10.3	21.4	49.4	28.0

* Lactic acid in tissues and blood is expressed throughout in terms of milligrams per cent.

** Lactic acid concentration of plasma, not included in computing average of blood lactic acid.

freezing of the tissue. In view of the well established fact that lactic acid forms rapidly in the excised brain (McGinty and Gesell, 1925; Haldi, 1932) we have selected a group of five experiments in both the control and injected series with an average time interval of 8 seconds each, so as to obtain as close an approximation as possible to the basal lactic acid level. The average lactic acid content of the control brain amounted to 51.8 mgm. per cent as compared with the average 54.3 mgm. per cent in the brain of the injected animals. Since this small difference between the control and experimentally treated brain lies within the limits of experimental error (which we have estimated to be 2 to 3 mgm. per cent) we are led to conclude that injection of bicarbonate had no effect on the basal lactic acid content of the brain.

Our data indicate, however, that after excision there occurred a more rapid formation of lactic acid in the brain of the injected animals. Twenty seconds after decapitation (making no allowance for the time to freeze the tissues), the brain of the control animals contained an average of 63.7 mgm. per cent lactic acid; 18 seconds after decapitation the brain of the bicarbonated animals contained 66.1 mgm. per cent. Interpolating the injected series to 20 seconds, the average is 4.7 mgm. per cent higher than the average of the control series. Here we observe a widening of the difference between averages which goes a little beyond the limits of experimental error. With a further increase in the time interval between decapitation and freezing of the tissue there occurred a greater increase in the percentage lactic acid content of the injected series over that of the controls. After 5 and 10 minutes' incubation the brain of the injected animals contained an average of 6 and 13.6 mgm. per cent, respectively, more lactic acid than the control brain. The greater accumulation of lactic acid in the brain of injected animals might have been due to the alkalinity of the blood trapped in the excised tissue. The tissue itself might also have become more alkaline as a result of the bicarbonate injection. In this connection we recall that Meyerhof (1921) found an augmentation of lactic acid production in excised muscle with increasing alkalinity of the surrounding medium. When the animals in our experiments were beheaded the blood was unquestionably strongly alkaline. In the experiments of Gesell, Krueger, Gorham and Bernthal (1930) the last blood sample taken more than an hour after injection of bicarbonate was markedly alkaline. Our results on incubated tissue suggest the possibility of a similar acceleration of lactic acid production in the brain *in vivo* following bicarbonate injection as a result of an augmented alkalinity of the blood, lymph and tissue cells. If this actually occurred, the fact that lactic acid did not accumulate in the brain above the basal level after injection would indicate a prompt diffusion of the excess lactic acid into the blood. The final solution of this problem, however, must look to more direct proof.

Maintenance of approximately the same lactic acid level in the brain after bicarbonate injection as before is extremely interesting in view of the fact that blood lactic acid rose more than 100 per cent above the pre-injection level. On *a priori* considerations one might expect either a depletion of lactic acid in the brain and other tissues, accounting for the rise in blood lactic acid, or an accumulation in the tissues, accompanying the rise in the lactic acid of the blood. The initial values in these experiments, it should be noted, represent the amount of lactic acid in the brain 15 to 30 minutes after injection and tell us nothing of the changes that might have occurred immediately following injection. It is possible that during or shortly after injection brain lactic acid rose to a higher level which was later reduced by diffusion into the blood but that this occurred appears improbable in view of the experiments of Gesell, Krueger, Gorham and Bernthal (1930). These workers found that the blood lactic acid continued to rise as late as 35 minutes after injection of bicarbonate, from which it is reasonable to suppose that in our experiments lactic acid was still mounting in the blood when the animal was beheaded. We are therefore inclined to believe that at no time was there any significant rise in the lactic acid content of the brain following bicarbonate injection.

Kidney. The kidney of the injected animals contained an average in 10 experiments of 45.5 mgm. per cent lactic acid 21 seconds after decapitation as compared with 29.8 mgm. per cent in the control kidney frozen 24 seconds after decapitation. This difference which amounts to a 53 per cent increase over the control kidney undoubtedly represents an actual accumulation in vivo of kidney lactic acid as a result of bicarbonate injection. From the data obtained in these experiments on the excised kidney no positive conclusions can be deduced as to the effect of bicarbonate on the rate of lactic acid formation. Unlike brain tissue the normal kidney showed a greater increase in lactic acid after 5 and 10 minutes' incubation than the kidney of the injected animals, 27.3 and 35.9 mgm. per cent respectively as against 22.5 and 29.9 mgm. per cent. At first thought it would appear that these results negate our tentative explanation attributing the greater increase in the brain of injected animals to the alkaline effect of bicarbonate on the excised tissue. In the kidney however we have a complicating factor in the superbasal accumulation of lactic acid which did not occur in the brain. The increase in intracellular lactic acid in the kidney might have counteracted the alkaline effect of the blood remaining in the excised organ. Furthermore, with a larger initial amount of lactic acid in the kidney after bicarbonate injection we might expect as a mass action effect a slower rate of lactic acid formation after excision.

Muscle. In six experiments the lactic acid in the muscle was 5.8 to 18.5 mgm. per cent higher after bicarbonate injection. In one experiment the muscle contained the same amount of lactic acid before and after

injection. The average increase in seven experiments after injection was 10.3 mgm. per cent. To determine whether manipulation of the animal was responsible for the increase in lactic acid, three control animals were handled in the same way as the injected animals and the sartorii analyzed for lactic acid. The difference between the lactic acid content of the two symmetrical muscles was well within the limits of experimental error. The values for the three pairs of muscles were 45.9 — 44.4; 67.3 — 65.0; 42.8 — 45.9 mgm. per cent. We are therefore led to conclude that the increase in muscle lactic acid is to be attributed to the bicarbonate.

The lactic acid values obtained for the resting muscle in these experiments were considerably higher than those found by Davenport and Davenport (1928) in guinea pig and rat muscle. This might have been due either to a species difference or to the effects of morphine-urethane anesthesia. We are inclined to attribute it largely to the anesthetic as lower values had been previously obtained in the vastus lateralis of un-anesthetized dogs (Haldi, 1932).

Urine. The urine collected in four experiments before injection contained 0.4, 19, 49.2 and 40 mgm. lactic acid. After injection the lactic acid in the urine from the same animals amounted to 4, 22, 30.6 and 74 mgm. respectively. The animal whose urine contained 0.4 and 4 mgm. lactic acid before and after injection apparently suffered an impairment of kidney function. Only 7 cc. of urine were found in the bladder immediately prior to injection. As far as could be learned no urine had been lost within the previous two hours. Twenty minutes after injection of 92 cc. bicarbonate solution the bladder contained only 20 cc. of urine. As a rule a relatively much larger amount was collected after injection. The animal with higher urinary lactic acid before injection was pregnant. In each instance the lactic acid in the urine before injection represents the excretion over at least two hours, whereas the urine collected after injection was excreted in 20 minutes. These observations are in line with the rise in urinary lactic acid excretion observed by Macleod (1918) after ingestion of sodium bicarbonate in therapeutic doses (amount of bicarbonate ingested not stated). Similar results were obtained by Macleod and Knapp (1918) with experimental animals following intravenous injection of sodium carbonate and by Jervell (1928) after administering to man 15 grams sodium bicarbonate per os.

Blood. A rise in blood lactic acid varying from 14.1 to 38.9 mgm. per cent, with an average of 28 mgm. per cent invariably followed bicarbonate injection. This represented an increase of 130 per cent over the preinjection level. It is worthy of note that after bicarbonate injection brain tissue in experiments 15, 16 and 17 contained a lower percentage of lactic acid than the blood. As the brain was dropped into liquid air within 5 to 7 seconds after decapitation the values obtained in these experiments

represent a close approximation to the basal lactic acid level of the tissue. A lower lactic acid concentration in brain tissue than in blood is also obtained if we plot a curve from the initial values of brain lactic acid in table 2 and extrapolate to zero time. In this way we obtain a basal value of approximately 42 mgm. per cent which is several milligrams lower than the average post-injection lactic acid content of the blood in the same experiments. Not anticipating the interesting relationship that later became evident in the distribution of lactic acid between brain tissue and blood, we unfortunately chose whole blood rather than plasma for analysis. Under both normal and experimental conditions plasma has been found to contain a higher percentage of lactic acid than whole blood (Hill, Long and Lupton, 1924; Eggleton and Evans, 1930b; Gesell, Krueger, Nicholson, Brassfield and Pelecovich, 1932a; 1932b). Three experiments were undertaken to determine whether there was a similar difference between plasma and whole blood lactic acid following bicarbonate injection. In each instance we found a higher percentage in the lactic acid of the plasma. In experiments 25, 26 and 27, table 2, in which plasma lactic acid was 50.2, 64.5 and 50.4 mgm. per cent, the whole blood contained 39.3, 54.7 and 44 mgm. per cent respectively. We are therefore justified in assuming that in experiments 13 to 24 the concentration of plasma lactic acid was higher than our figures for whole blood. Accordingly we would expect brain lactic acid to be lower than plasma lactic acid by more than 7.4 mgm. per cent, the difference in our experiments between the lactic acid content of whole blood and brain lactic acid extrapolated to zero time. Under various conditions other tissues have also been found to contain a lower percentage of lactic acid than the plasma. Eggleton and Evans (1930b) reported that muscle taken from the fore limbs of the dog after stimulation of the hind limbs contained more lactic acid than blood plasma. Gesell, Krueger, Nicholson, Brassfield and Pelecovich (1932a) found that lactic acid concentration in the testicle was lower than in plasma. After administration of low oxygen and injection of methylene blue the percentage of lactic acid in muscle which was originally higher than in plasma was reduced considerably lower than that of plasma (Gesell, Krueger, Nicholson, Brassfield and Pelecovich, 1932a, b).

Lactic acid concentration in the kidney after bicarbonate injection approximated that of whole blood. Since plasma contained more lactic acid than the whole blood in the analyses referred to above, we are led to conclude from the data of these experiments that in the living animal plasma lactic acid was at a higher level than in the kidney, suggesting that the rise in kidney lactic acid concomitant with the increase in blood lactic acid was due to diffusion from the blood into the kidney. In experiments 17 and 22 the lactic acid concentration in the kidney was higher than in the blood but it is possible that lactic acid in the plasma may have been at a

higher concentration than in the kidney, as we found in one instance approximately thirty per cent more lactic acid in plasma than in whole blood. Furthermore, it should be noted that the kidney was frozen approximately 25 seconds after excision, consequently our lactic acid values were higher than the basal concentration and, secondly, the blood was drawn 4 to 5 minutes before decapitation of the animal. If the blood had been taken simultaneously with excision of the kidney it would have probably had a higher lactic acid concentration than our sample. (Cf. Gesell, Krueger, Gorham and Bernthal, 1930.)

DISCUSSION. While these experiments, confirming the observations of other workers, show clearly that bicarbonate injection produces a marked increase in blood lactic acid, it is interesting to note that Bock, Dill and Edwards (1932) were led to believe from a recent investigation that in man there is no mechanism operating, as in experimental animals, to increase blood lactic acid in response to a rise in blood bicarbonate. In their experiments in which four normal men ingested 20 gram doses of sodium bicarbonate at 9 a.m., 11 a.m. and 2 p.m., blood samples drawn at intervals of one or several hours showed an increase in bicarbonate but not in lactic acid. It is important to note, however, that here we have a set of conditions entirely different from those in our experiments. Assuming that the weight of the subjects was in the neighborhood of 70 kilos, the amount ingested approximated 850 mgm. bicarbonate per kilo body weight, some of which was probably not absorbed. Absorption into the blood took place over a period of several hours, whereas in contrast with this procedure, approximately 1.7 grams bicarbonate per kilo body weight were introduced directly into the blood stream of our animals within a few minutes.

Acute experiments of this nature on animals we believe might reveal fundamental mechanisms not detected in the experiments by Bock, Dill and Edwards. With slow administration of bicarbonate and gradual absorption into the blood stream an increased production of lactic acid could perhaps escape unnoticed if it were diffusing into the kidneys and being eliminated in the urine. It is of course possible that the lactic acid producing mechanism comes into play only as an outer line of defense against alkalosis when the bicarbonate of the blood has reached dangerously high levels.

One experiment reported by Bock, Dill and Edwards in which a larger amount of bicarbonate was administered confirms our results. A dog weighing 13 kgm. was given 225 cc. of a 10 per cent sodium bicarbonate solution of which 60 cc. were discharged as vomitus leaving a total of 165 cc. or 16.5 grams, equivalent to 1.27 grams bicarbonate per kilo body weight. Thirteen minutes later the lactic acid of the blood rose from 17.4 to 24.5 mgm. per cent, an increase of 50 per cent over the original amount

which can not be regarded as insignificant. In the course of 3 hours and 40 minutes 848 cc. (84.8 grams) were introduced into the stomach of which 563 cc. (56.3 grams) or 4.33 grams per kilo were retained, 285 cc. having been lost in vomitus. The lactic acid of the blood at the end of this time rose from 17.4 to 47.4 mgm. per cent.

We may now raise the question, what is the underlying mechanism accounting for the increase in blood lactic acid following bicarbonate injection? Eggleton and Evans (1930) finding that either under-ventilation or injection of hydrochloric acid lowered blood lactic acid whereas sodium bicarbonate or over-ventilation augmented it, concluded that "the important factor is not the bicarbonate concentration but the H-ion concentration or what amounts to the same thing, the acid-base ratio." The same opinion had been previously expressed by Anrep and Cannon (1923) and adopted also by Long (1924). The artificial respiration experiments of Gesell, Krueger, Gorham and Bernthal (1930) reveal that there is no invariable uniform relationship between changes in blood acidity and blood lactic acid. Sodium bicarbonate led to a marked alkalinity of the blood with a concomitant increase in blood lactic acid whereas hemorrhage and injection of sodium cyanide produced a rise in both the acidity and lactic acid of the blood. Johnston and Wilson (1930) also found an increase in blood lactic acid associated with increased acidity after hemorrhage in normal unanesthetized dogs. In a series of experiments in which the animal's ventilation was under normal physiological control, Gesell, Krueger, Nicholson, Brassfield and Pelecovich (1932) found that when sodium cyanide was injected, blood lactic acid rose consistently while the blood at first turned alkaline and then became acid. More recently, in the experiments referred to previously, Bock, Dill and Edwards (1932) showed that lactic acid concentration in the blood of resting man is not related simply to shifts in hydrogen ion concentration. Alkalosis induced by ingestion of sodium bicarbonate and acidosis produced by ammonium chloride had no effect on the concentration of blood lactic acid.

The lack of uniform relationship between blood acidity and blood lactic acid inclines us to look elsewhere than to changes in hydrogen ion concentration to account for the increase in blood lactic acid in our experiments. It is possible, as suggested by Gesell, Krueger, Gorham and Bernthal (1930) that the increased pressure of HCO_3 ions in the blood following bicarbonate injection results in their diffusion into the tissues and the replacement of lactate ions which in turn migrate into the blood. As a consequence of this exchange lactic acid in the cells would be reduced or depleted (at least momentarily) followed by the formation of new lactic acid and the restoration of the initial lactic acid level in the tissues. A somewhat similar hypothesis might be suggested on the basis of mass action, attributing the increased lactic acid formation to a shift in the equilibrium between the

precursor substance and lactic acid by reason of diffusion of bicarbonate into the cells. The original equilibrium disturbed by reaction of base with the intracellular lactic acid would tend to be restored by the formation of new lactic acid. Sodium lactate diffusing out of the cells would augment the blood lactic acid which in turn might diffuse into tissues (as the kidney) with a lower lactic acid level. Throughout this discussion we have kept in mind that as yet we do not know in what tissues the additional lactic acid is formed after bicarbonate injection. Finally, serious consideration should also be given to Macleod's suggestion (1918) of the possibility of a fundamental mechanism operating to counteract the bicarbonate alkalosis. Without more complete information all these explanations are highly hypothetical. A final answer to the problem must await further investigation.

SUMMARY

The effects produced by intravenous injection of sodium bicarbonate on the lactic acid content of blood and tissues have been investigated.

Following bicarbonate injection brain tissue immersed in liquid air 8 seconds after decapitation contained approximately the same lactic acid concentration as the control brain. The kidney of bicarbonated animals contained an average of 25.7 mgm. per cent more lactic acid than the control kidney. Lactic acid in the muscle after injection in 6 experiments was 5.8 to 18.5 mgm. per cent higher than in the symmetrical muscle in the same animal before injection. In one experiment there was no change in lactic acid concentration after bicarbonate. The average increase in the seven experiments was 10.3 mgm. per cent.

In the excised brain of injected animals there occurred a slightly greater accumulation of lactic acid after 5 and 10 minutes' incubation than in the control brain. Conversely, there was a relatively larger increase in the lactic acid of the control kidney after incubation than in the kidney of bicarbonated animals. An explanation of these results was proposed.

Bicarbonate invariably produced a marked rise in blood lactic acid within a few minutes after injection. These results confirm previous observations by other workers. The average increase in eleven experiments was 28 mgm. per cent or an increase of approximately 130 per cent over the pre-injection level.

The concentration of blood lactic acid which normally is 20 to 30 mgm. p. c. lower than in the brain, after bicarbonate injection rose to a higher level than brain lactic acid in three experiments in which the brain was frozen 5 to 7 seconds after decapitation. Extrapolation of the curve plotted from all the brain lactic acid values following the injection gave a basal level of brain lactic acid below the average blood lactic acid content after injection.

The lactic acid concentration of the kidney which rose concomitantly with that of the blood after injection of bicarbonate, in most of the experi-

ments remained below the lactic acid level in the blood. Analyses of the urine in several experiments indicated that the bicarbonated animals excreted more lactic acid than the uninjected animals.

The mechanism accounting for the increase in blood lactic acid produced by injection of bicarbonate was discussed.

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BIOLOGICAL DIFFERENCES IN RESPONSE OF THE FEMALE MACACUS MONKEY TO EXTRACTS OF THE ANTERIOR PITUITARY AND OF HUMAN PREGNANCY URINE¹

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Due to the wide dissemination of the early observations on the gonad stimulating action of anterior pituitary implants and extracts, and administration of human pregnancy urine, either as a whole product or in the form of extracts, it has become generally and too often uncritically accepted that these two substances were identical. As work has progressed in the hands of a score of investigators, and other test forms have been used, the evidences of differences in biological response between extracts from the two main sources have multiplied (Wallen-Lawrence and van Dyke; Collip et al.; Evans, Meyer and Simpson, among others). Both Fels and Phillip very early expressed doubt that the glandular and urinary substances were identical.

The observations of Schockaert (1933) on the differences in response of the male rat to injections of pregnancy urine and of basic extract of the anterior pituitary were made before those of the writer (Engle, 1932a) who contrasted the effects of the A. P. pyridine extract of the gland and of PU. Schockaert's extensive and adequately presented data leave no room for doubt as to decided differences in response of the male gonad to extracts from these two sources.

The extensive data of Domm, Riddle, and of Smith and Leonard on another test form, the immature male fowl; the observations of Reichert et al., Collip, and of Smith on the hypophysectomized rat; and Schockaert's previous work on the duck and the fowl form a rapidly increasing pyramid of evidence which calls again into question the premature conclusions regarding the identity of the gonad activators derived from the anterior pituitary itself and from human pregnancy urine.

The present report is limited to the relation of the accepted gonadokinetic factors of the anterior pituitary and of human pregnancy urine in relation to the functioning and structure of the ovary of the macacus

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monkey. A large group of animals have not been run at any one time. Rather each animal has constituted a single experiment, and due to the length of time required for treating an animal as well as the longer time required for sectioning and studying serial sections, relatively few series may be completed within a year.

Effects of implants of rat pituitaries. In the first attempts to secure ovarian activation in monkeys implants were used. Implants of rat pituitaries, either from male or female animals or both without distinction, were given to seven small monkeys.

Allen (1928) had implanted subcutaneously the anterior pituitaries of four monkeys into a 3465 gram adolescent female Macaque. Hartman and Squier (1931) report the result of implanting the hypophyses of castrated adult male pigs in Macaques. Each of the recipient monkeys had been menstruating previous to this experiment, and were physiologically more mature than Allen's monkey, and much older than those of the present series.

The smallest monkey of the present series was an infant, only 1800 grams (My 27), the largest not yet entered adolescence, 3300 grams (My 51). The best ovarian response was obtained in a 2500 gram animal (My 43) which, over a period of 19 consecutive days, was given a total of 111 rat anterior lobes from male animals. The ovaries were more than ten times normal size, no small follicles remaining, all being in cystic degeneration, with no luteinization. The typical oestrin effect on the perigenital skin appeared early (10th day).

One animal (My 27), 1800 grams, represents a second type of response. This, the smallest animal used, was treated for 26 consecutive days with 145 rat AP from male animals. The perigenital skin showed an excellent response, but the effect was lost during treatment. The ovaries were not large, 82.8 and 68.5 mgm., and showed no luteinization.

The other animals, 2500, 2650, 3300 grams, showed little or no response of sex skin, and very slight ovarian response even though they were treated with rat AP from both sexes, mostly females, for 30, 10 and 16 days respectively. The rat-implanted monkeys in two cases gave spectacular response of the sex skin, and little or none in the others. Tentatively this refractory condition may be considered to be due, in part, to the state of the ovary of the host, which depends among other things on age. Two cases have been observed in which there was a failure of response of the genital skin after treatment with AP extract. This indicates that the ovary of an individual animal may sometimes be non-reactive or refractory.

Response to extracts from anterior pituitary gland. The action of the pyridine extract of anterior pituitary on the ovary of macacus monkeys has been reported in two brief papers by Hisaw and his associates (Hisaw, Fevold and Leonard, 1931; Hisaw, Hertz, Hellbaum and Fevold, 1932).

The data accumulated from Hisaw's laboratory represent extensive and careful analyses of this problem. The present report is a confirmation of their investigations, which in our work have been extended to include also the action of pregnancy urine.^{2, 3, 4}

Since the age of these monkeys is unknown, weight and the usual external signs afford the only criterion of development. However, it is known that considerable variation in the degree of maturity of the animals is encountered when weight serves as a criterion, for certain animals of 3300 gram weight may be more nearly mature than others of 3800 gram weight. Size of ovaries and uterus, measured at an exploratory operation, gives considerable assurance of control measures. This has not been done in all instances due to complications of other experimental procedure.

A few typical protocols will serve the present purpose.

My 65—3000 grams received a total of 3.52 grams equivalent of dried sheep pituitary powder administered subcutaneously, over a period of 13 days. Genital reddening and some swelling developed from the 8th to 13th days. Animal became ill from unknown cause, sacrificed two days after last injection. Ovaries were the largest obtained or seen in so young a monkey, although smaller than those reported by Hartman and Squier.

Right ovary $13.9 \times 8.8 \times 7.0$, weight 514.1 mgm.

Left ovary $13.9 \times 8.8 \times 7.6$, weight 515.2 mgm.

Uterus infantile, "pencil size."

The ovary is a mass of cysts with few small or medium sized follicles remaining. Moderate amount of granulosa remains on periphery of the follicle, 6 to 8 cell rows. Except in two follicles there is no change which could be interpreted as a transition toward luteinization, and this change only in a small localized area in the former cumulus oöphorus. The ova are cytolized in all of the cystic follicles. Many large follicles are cystic in condition only and not in size. These have irregular contours, show the presence of characteristic pycnotic wandering cells which are well known in atretic follicles of all forms, with very few cell-layers of granulosa.

The ovaries of this animal proceeded with great rapidity to cystic degeneration. The degree of oestrin effect, however, as evidenced by the moderate development of the sex skin and by the lack of size and tonus of

² The commercial preparations used in these studies, Antuitrin S and the sheep pituitary powder were furnished by Parke, Davis & Co., through the interest and courtesy of Dr. O. P. Kamm. The pregnancy urine was obtained from the out patient department of Sloane Hospital for Women, Dr. B. P. Watson, Director.

³ The writer wishes to express his thanks to Dr. M. C. Shelesnyak for assistance in all surgical procedures involved in this study.

⁴ The extracts of the gland or of pregnancy urine are not assayed in rat or mouse units. A minimum degree of potency is determined by such tests, but in none of these instances have the unit values been of much significance, since maximal effects have been sought. The commercial preparations are quoted for the same reason at their cited concentration.

the uterus, was much below that of other animals with smaller follicles such as My 106 (see below).

My 89—4450 grams, an adult female, previous history unknown. A corpus luteum of ovulation was present in one ovary, indicating previous cycles. Both ovaries were left intact. It was treated subcutaneously with a pyridine extract for 13 days. Due to long standing, this preparation had lost much potency, and was administered in unusually large quantities (equivalent to 19 gm. dried powder). By the seventh day the previously faded sex skin had become a deep reddish-purple, with vulvar edema accompanied by pygial pachydermatous rugosities and roughening of the skin of the medial surface of the thighs. Bilateral ovariectomy performed on the 14th day. The right ovary measured $12.7 \times 10.3 \times 8.1$ mm., the left $13.2 \times 10.4 \times 9.3$. Weight of both ovaries, 1.112 grams (fig. 4).

Cystic degeneration is not as marked as in *My 65* ♀ but all follicles have responded to the follicle-activator (fig. 4). Many of these small cysts are denuded of granulosa while in other follicles the granulosa is still intact but undergoing atretic degeneration. There are patches of unmistakable lutein tissue in the thecal-granulosar border but in no instance has the follicle become transformed into even a loosely organized corpus luteum. The right tube, removed at the time of ovariectomy, shows no progestational modification, although the tubal mucosa is more sensitive to this stimulus than the endometrium.

My 106—3650 grams, no previous ovulation. Previous menstrual history was unknown. Six days' treatment by subcutaneous injection of pyridine extract, followed by 3 days' intravenous injection; treatment twice daily, extract equivalent to six grams dried sheep powder. Bilateral ovariectomy on 10th day.

Right ovary $10.2 \times 5.9 \times 4.3$ mm., weight 154.5 mgm.

Left ovary $9.5 \times 6.2 \times 4.6$ mm., weight 156.3 mgm.

Microscopic—all follicles built up, some small cystic degeneration. Granulosa borders intact, many mitotic figures in granulosa. Lutein changes pronounced in theca—many cells in granulosa showing large nucleus and cytoplasm, quite luteoid in character. Many eggs cytolized. Most of smaller follicles have undergone definite lutein progression with characteristic spindle cell migration of the young primate corpus luteum. The large follicles do not show this type of change. The mucosa of the uterine tube does not show progestational proliferation.

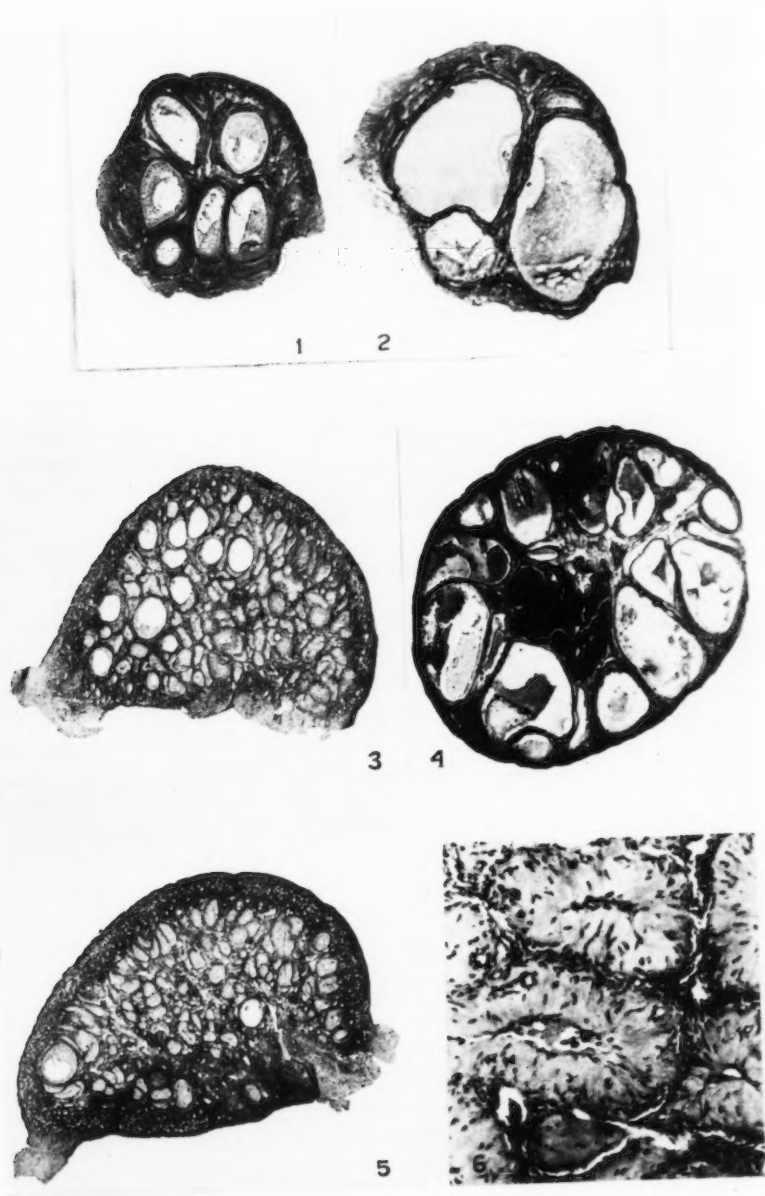
Figs. 1 and 2. Right (control) ovary and left (treated) ovary of *My 136*, treated for 10 days with anterior pituitary gland extract (*vide* protocol).

Fig. 3. Right ovary of *My 110*, after treatment with the gonad-stimulating fraction of pregnancy urine for 9 days (*vide* protocol).

Fig. 4. Left ovary of *My 89* treated for 13 days with anterior pituitary gland extract (*vide* protocol).

Fig. 5. Ovary of *My 113* (3000 gm.) after treatment with gonad-stimulating extract of pregnancy urine (Antuitrin S) intravenously for 21 days, total dosage 4200 units. No flushing of sex skin occurred during treatment.

Fig. 6. High power of section illustrated in figure 5, showing the type of hyalinization of smaller follicles.



Figs. 1 to 6

My 136—3400 grams, previous history unknown. Right ovary removed $5.0 \times 4.2 \times 3.1$ mm. 45.2 mgm. No large follicles in left ovary. Ten days' treatment water soluble AP injected subcutaneously; 21 cc. = 7 grams dried sheep powder. Left ovary removed on 11th day. Left ovary $6.8 \times 4.7 \times 4.0$; 92 mgm.

Usual stimulation of all follicles, no luteal change, considerable degree of nuclear degeneration and cytolytic change in egg cells. Figures 1 and 2.

These representative protocols taken from 20 cases similarly treated with anterior pituitary extract are sufficient to indicate the results, which are uniform. The age and condition of the monkeys are different, and the amount of the active principle and length of treatment vary. Within the limitations of these experiments, however, the results are uniform. The two animals which were treated with an extract of proven potency and which showed no change of the sex skin nor follicular development are the exceptions in this series. The reason for a lack of response is not understood. One or both ovaries of each of these 20 animals have been serially sectioned at 8μ and studied. Growth of all or nearly all follicles, which may easily be forced into cystic degeneration, occurs. Hemorrhagic follicles may occur, usually only one or two in an ovary. Lutein and luteoid changes occur in the borders of many of the larger follicles, but no instance of complete lutein obliteration of the follicular cavity has been seen in any of these 20 animals after subcutaneous injections as is so readily apparent in the rodent after treatment with the same substance. Completely luteinized ovaries obtained under other conditions will be reported later.

The smaller follicles do show a greater degree of luteinization than the larger ones. Lutein changes in cystic follicles are rare.

During the period of adolescence certain easily recognizable changes of the circumgenital area are known to occur. The changes in this area, known as the "sex skin," are now clearly referable to this period of adolescence, due to the numerous and accurate observations of Hartman. According to the same observer such extensive changes do not normally occur in fully mature females. Allen's early experiments clearly show that these phenomena are due only to oestrin, a relatively small amount of oestrin being needed to induce the change in a castrate (total dosage of 25 to 80 R.U.) (Allen, 1927).

The successful use of the aqueous pyridine extract of sheep AP in macacus monkeys has been reported by Hisaw, Fevold and Leonard (1931). The resultant follicular growth, cyst formation and marked change in the sex skin were constant. Courier, Kehl and Raynaud (1929) reported somewhat similar response in one monkey. Saiki (1932) also used an anterior pituitary extract which produced certain follicular changes, but these not so extensive as those of Hisaw and his associates nor those of the author.

Novak and Kun (1931) record protocols of 3 females and 1 male Rhesus

monkey which were treated with "Anteron." This material consisted of two preparations, one from pregnancy urine, the other from dried anterior lobe substance. In the protocols the source of the particular extract used for a given animal is not given. However, since it is explicitly stated that the hormones from these two sources are the same, and since the term "Anteron-Mengen" was used in the protocols, the impression is obtained either that the substances were given as pooled samples, in which case their results would be uniform with those obtained in this laboratory, or that they were given intercurrently. Of the three females weighing 3060 grams, 4300 grams and 4000 grams, the first showed no change of sex skin, the second showed the beginning of change on the 41st day of injection, and the third, swelling and reddening on the 23rd day. In our experience, these changes have usually occurred after treatment with AP in a degree considerably greater than is obtained when giving oestrin alone. Here also, the highest degree of turgosity and pachydermatosis occurs in animals under 4000 grams, although the coloring is very striking even in larger females. These changes in the sex skin of the juvenile or adolescent *Macacus* female are due solely to the action of oestrin. This may be administered directly or its action may be elicited by means of follicular stimulation during administration of an active principle of the anterior pituitary. As in the experience of Allen with oestrin, and that of Hisaw, Fevold and Leonard, and the writer with anterior pituitary extract these changes in the sex skin are constant and dependable phenomena, which may be called forth with a relatively low degree of oestrin activity.

Response to derivatives of pregnancy urine. Previous to the experiments on the action of the pyridine extracts of the anterior lobe a commercial "luteinizing hormone" had been used on three monkeys. This was, according to advice received at the time, a pregnancy urine derivative.

Three monkeys, My 11 (2400 gm.), My 19 (2150 gm.) and My 28 (2050 gm.) were treated with this preparation. The protocol of My 11, the largest of this group, is given.

April 7, 1931, "luteinizing hormone" (Parke-Davis & Co., 50 R.U./cc.) given twice daily subcutaneously for 17 days total 50 cc. (2500 R.U.). Laparotomy—right ovariectomy—right ovary 8.2 mm. long, 107.6 mgm. Follicles few and small, none over 2 mm. greatest diameter. Many C.L. atretica. At no time during the treatment did the animal show any oestrin-effect on the sex skin.

The other animals showed similar lack of ovarian or cutaneous response, and it was thought that the animals were too young to respond to the treatment. This type of extract was not used again for nearly a year, when larger monkeys were examined. In the meantime it had been determined that the age of the monkey was not too important, as juvenile

animals of 1800 to 2000 grams would respond to treatment with an appropriate activator, though the response is neither so constant nor uniform as in 3000 gram animals.

Two preparations were used in this series: one, the commercial preparation Antuitrin S, a pregnancy urine derivative, the other a preparation made after Zondek's method of alcoholic precipitation, ether washing and water solution. The urine consisted of pooled samples of pregnancy urine of the 2nd to 5th month. While each sample of extract was tested for potency the rat unit value was not determined in most cases, the dosage being given in liters equivalent of whole urine.

Protocol of My 112—3500 grams. Received concentrated extract from 1710 cc. of pregnancy urine over a period of 22 days, administered subcutaneously. No change in color, condition or texture of sex skin. Ovaries at termination of experiment minute, 38 and 47.7 mgm., consisting of medium sized follicles, which appear to be not active histologically. Increase in number of small hyaline bodies.

Protocol of My 110—3900 grams. Antuitrin S administered intravenously for 9 days, total dosage 17 cc. (1800 M.U.). On completion of experiment ovaries weighed 100 and 129 mgm. No change in sex skin had occurred. Follicles small, few normal. All of the small and medium sized follicles had undergone hyalinization. Figure 3.

Protocol of My 111 ♀—3150 grams Antuitrin S administered intravenously for 22 days, total dosage of 40 cc., or 8000 units. Left ovary removed, 99 mgm. No change in sex skin. Condition of ovary as in My 110, 112.

Ovaries of ten animals treated with pregnancy urine derivatives have been studied, while observations on external response have included a much larger number. Previous use of such preparations by other investigators has been limited.

Hartman, Firor and Geiling did not stress ovarian reactions to treatment since their work is of importance in the related problem of uterine bleeding.

In brief reports, the statement has been made (Engle, 1932c, 33) that, whereas a relatively low degree of oestrin stimulation will cause change of the sex skin in a pre-pubertal monkey, either with AP or oestrin, this very significant change has not occurred in animals which have been treated with the preparations of pregnancy urine which are known to be activators of the rodent ovary. The follicles show no growth after treatment with pregnancy urine fractions; little or no oestrin is produced. Signs of atresia are present in the larger follicles, but the most marked response is the general hyalinization of the smaller follicles, into a type of pseudocorpora lutea atretica seen occasionally in ovaries of immature and mature monkeys (figs. 5 and 6). Such bodies are present in human ovaries and appear to be identical with the ones illustrated here. The difference is that whereas in the normal ovary one or two of these bodies occur, in the treated animal they occupy almost the entire follicle-bearing portion of the ovary.

This change in the morphology of the ovary has not resulted in a significant weight increase. The relatively large increase in the size of ovaries treated with glandular preparations has been noted above.

The age and sexual condition of the sub-adult monkey seem not to be important, as animals as large as 3900 grams have not responded to this type of treatment, and the ovarian condition is similar to that of a 3000 gram monkey. A single large animal, My 96 (4900 gm.), was treated for 32 days without showing any change in sex skin. The ovaries of this animal were not examined.

Neither do these hyalinized bodies, nor their transitional forms have any morphological similarity to corpora lutea, either to those induced by hormone treatment nor those resulting from normal ovulation in the monkey⁵ or in the human. Other pelvic change, especially the prolonged but often intermittent uterine bleeding during treatment have been a result of pregnancy urine only (Engle, 1932).

DISCUSSION. The pituitary body is a glandular entity common to all vertebrates. In numerous forms it has been shown to possess gonad-stimulating qualities.

The substance in human urine of pregnancy, whatever its source, which causes the typical ovarian changes in the rat, mouse and rabbit is not found normally in any other animal form. A similar though possibly not identical substance is present in the blood serum of pregnant mares during a portion of the gestational period (Cole and Hart). The substance referred to, so far as we know, is excreted in large amounts only by the human, and probably the great apes during pregnancy, and by the human in certain other conditions (genital tumors, after the menopause, etc.). It has been shown not to be a part of the physiological economy of pregnant macacus monkeys (Allen, Maddux and Kennedy, 1931; Snyder and Wislocki, 1931).

The present series of observations is extensive enough to justify the statement that this active principle which causes follicular growth with oestrin secretion adequate for induction of oestrus and mating, followed by the development of secretory lutein bodies adequate to maintain uterine deciduoma (Shelesnyak) in the rodent's ovary, does not so operate in the macacus monkey, when used without the addition of any other substance, or without having been preceded by other substances.

While further details are being studied, it must be borne in mind that in the sub-anthropoids at least, this substance from human pregnancy urine is a foreign factor, and it is possible that the responses are caused by

⁵ The writer is indebted to Dr. Carl Hartman for the privilege of examining slides of normal corpora of ovulation in which the age of the body was accurately known.

active principles which are not natively physiological in the experimental animals.

SUMMARY

1. This paper represents a preliminary statement on the reaction of the ovaries of macacus monkeys which have been studied during a three year period. Ovaries of 37 animals from various series of experiments have been studied. The animals ranged in weight from 1800 grams to 5150 grams.

2. Extracts of the anterior pituitary gland cause follicular growth and cystic development in the monkey ovary as shown by Hisaw et al. Six to ten days after initiation of treatment in sub-adult animals and usually in immature monkeys, ovarian activation is evidenced by changes in the sex skin; reddening, turgosity, pachydermatous foldings. Adult animals show a lesser degree of external response.

3. Slight theca lutein changes are produced by this treatment with *subcutaneous injections*, but no typical lutein bodies have been formed by this method.

4. When using the gonad-stimulating substance from pregnancy urine no such response has been obtained in immature, sub-adult or mature female monkeys.

5. Since no activation of the sex skin has occurred with subcutaneous or intravenous injections for as long as 32 days, it is concluded that, as used, there is no follicular activation.

6. Instead of follicular growth there appears to be a cessation of ovarian activity, with atresia of large follicles and hyalinization of the small follicles.

7. It is emphasized that this substance derived from human pregnancy urine is not known to be present in macacus monkeys during any physiological state, and, as used, is a depressant rather than a stimulant of follicular development in the monkey ovary.

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THE INFLUENCE OF STRETCH ON THE LENGTH OF SURVIVAL OF ISOLATED MUSCLES

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Several years ago it was found by the author (1) that, in adult toads, a whole muscle, transplanted from the limb into the back, will remain in perfect functional condition, provided care has been taken to incorporate the graft under a certain stretch. Since stretch thus appeared to be essential for the permanent maintenance of excitability and contractility in *grafted* muscles, the question arose whether in *isolated* muscles stretch might not have a similar influence on the maintenance of excitability and contractility. The experiments described below were undertaken to solve this question. The results unmistakably establish the existence of influences of this sort: *If an isolated muscle is subjected to persistent stretch, there results a prolongation of the period during which the muscle is found to be excitable by direct electrical stimulation.* We may call this period "survival period." The experiments consisted in comparing the "survival period" of stretched and of loose muscles.

MATERIALS AND METHODS. The experiments were done on adult frogs (*Rana pipiens*) of about 50 grams' body weight, during the months of November and December (ether anesthesia and crushing of the brain). The muscles were carefully prepared; injured muscles were never used for experimentation. After isolation, the muscles were immediately transferred into aerated Ringer's solution. Silk threads were tied to the tendinous ends of each muscle. As holder for both the stretched and the loose muscles, a rectangular glass frame, about 7 cm. in length, was used. The shorter sides of the frame were provided each with a pair of glass hooks on which the free ends of the silk threads were fastened. Each frame held a pair of corresponding muscles from the right and left legs. Larger frames containing 3 or 4 pairs of muscles were also used. The frames carrying the muscles were put in Petri dishes containing about 50 cc. of Ringer's solution which, in some cases, was renewed once or twice during the course of the experiment. A few experiments were done in moist chambers instead of in Ringer's solution. The dishes were kept at room temperature (around 19°C.).

At intervals, the excitability of the isolated muscles was tested by single

induction shocks. For this purpose the muscles were temporarily removed from Ringer's solution. The threshold intensity of the stimuli was determined by varying the coil distance. Since different parts of the same muscle, especially during the last hours before death, were found to differ very markedly in their thresholds, a strict definition of what had to be considered as the characteristic threshold value was required: this was taken to be the *lowest* threshold intensity that occurred in any part of the muscle. Consequently, a muscle in which part of the fibers had already become unexcitable, was still recorded as excitable, as long as any fibers in it responded. Only after the muscle had entirely ceased to react to the strongest faradic stimulation was it considered as dead. In seven experiments the muscles were connected with isometric levers and allowed to write on a kymograph during the excitability tests. In all the other experiments, however, the responses were directly observed.

Eight different sorts of muscles were used. These are (2):

M. gastrocnemius (plantaris longus)	Abbrev.: G
M. semitendinosus	Abbrev.: Se
M. sartorius	Abbrev.: Sa
M. tibialis anticus longus	Abbrev.: T
M. ilio-fibularis	Abbrev.: If
M. flexor digitorum brevis superf.	Abbrev.: Fld
M. peroneus	Abbrev.: P
M. coraco-radialis	Abbrev.: Corr

Whenever the survival periods of a loose and a corresponding stretched muscle differ in favor of the latter, we call this time difference "positive stretch effect."

EXPERIMENTS. Six different sorts of experiments were performed:

A. Both muscles loose (control experiments to determine how much corresponding muscles under identical conditions may differ in their survival period). Series "*LL*."

B. One muscle loose, the other stretched. Series "*LS*."

C. Both muscles stretched. Series "*SS*."

D. At first, both muscles loose. Later, after an interval varying in different experiments between 11 and 43 hours, one of them stretched. Series "*LL* \rightarrow *LS*."

E. At first, both muscles stretched. Later, after an interval varying for different experiments between 23 and 41 hours, one of them loosened. Series "*SS* \rightarrow *LS*."

F. At first, one muscle loose, the other stretched. After an interval varying for different experiments between 17 and 41 hours, the stretched muscle was released. Series "*LS* \rightarrow *LL*."

Experiments D, E, and F were done in order to ascertain whether stretch exhibits its influence permanently during the whole survival period or

whether its influence is restricted either to the beginning or to the end of this period. In experiments D and E, both muscles were at first kept under the same conditions, either both loose or both stretched, and the condition to be tested—one muscle loose, the other stretched—was established only during the later stages of the survival period; whereas, in experiment F, the critical condition was maintained only during the early stages of the survival period.

The total number of experiments was 154, each involving one pair of corresponding muscles. Fifty-seven of these experiments had to be discarded because no record of the exact time of death of either muscle was obtained. The remaining 97 experiments are listed in table 1.

Isolated muscles which are kept loose are usually found to die on the second or third day after isolation (in Ringer's solution). There is a wide range of variability in the survival period of muscles taken from

TABLE 1

SERIES	SORT OF MUSCLE*								NUMBER OF EXPERIMENTS
	G	Se	T	Sa	If	P	Fld	Corr	
LL	3	2	3	1	2	2	—	—	13
SS	—	1	—	1	—	—	—	—	2
LS	16	6	10	5	4	5	1	3	50
LL → LS	5	2	3	1	2	—	2	—	15
SS → LS	2	3	2	1	2	—	2	—	12
LS → LL	3	—	1	—	—	—	1	—	5
Number of experiments.....	29	14	19	9	10	7	6	3	97

* For explanation of abbreviations cf. text.

different animals, but no attempt to pursue this interesting question has been made in the course of the present experiments. There is much less variability in the survival period of different muscles of the same animal than there is for the same muscles of different animals. The death of all the muscles of one and the same animal occurs within a comparatively narrow time limit, the mean survival period of the muscles of an animal being an individual characteristic of that animal or, at least, of its condition at the time of dissection. The situation is clearly illustrated by the graph in figure 1. Here the time of death of the isolated muscles of 14 animals is indicated, and it can readily be seen that the death marks of the different muscles of each animal are rather closely grouped around certain average time values which differ between different animals. If we consider that the smallest and the largest muscles of any one animal in these experiments differed in size as much as 1:20, we realize that the survival period of a muscle does not depend on its size.

The comparatively small variability in the survival periods of *different* muscles of the same animal is still considerably higher than that found in comparing two *corresponding* muscles of the same animal. In this latter case, the difference is almost negligible. This is evidenced by the experiments of:

A. Series LL. Thirteen pairs of corresponding muscles were isolated and both muscles of each pair were kept loose. The greatest observed difference in the survival period of such pairs was $2\frac{1}{2}$ hours, the average for all experiments being 1.1 hour.

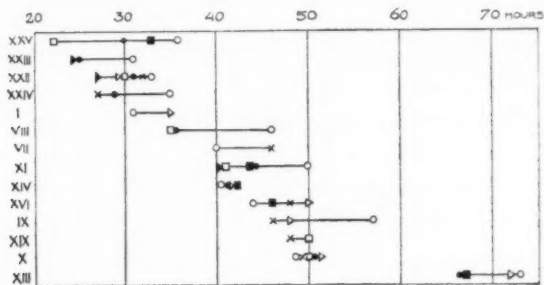


Fig. 1. Showing the death marks of the various isolated muscles of 14 animals. The time of death is given in hours after isolation. The Roman figures are the protocol numbers of the animals. The range of variability in the survival period of different muscles of the same animal is indicated by a line connecting the death marks of the shortest and the longest surviving muscles. The marks represent:

- M. gastrocnemius
- × M. semitendinosus
- M. sartorius
- M. tibialis ant. long.
- △ M. iliofibularis
- M. flexor digitorum brev. superf.
- ▲ M. peroneus

The fair coincidence of the values for corresponding muscles of the same animal justifies using one muscle of a pair as control for the other, as was always the procedure in the subsequent experiments.

B. Series LS. In this series, comprising 50 experiments, the prolongation of the survival period of muscle by stretch is clearly demonstrated. One muscle of each pair was tied to the frame under some stretch, whereas the other muscle was kept loose as control. In a few experiments, the intensity of stretch was measured by a weight or by the excursion of the isometric lever. Since no definite relation between the amount of stretch applied to the muscle and the prolongation of the survival period was observed in the preliminary experiments, recording of the intensity of

stretch was omitted during the subsequent experiments. To give some idea of the amount of stretch applied, it may be stated that it was always such as to extend the muscle a good deal beyond the maximum possible physiological extension without approaching too closely the limit of elasticity.

In two out of the fifty cases, the loose muscle survived the stretched one; in the first of these two cases (P) the difference was about 2 hours, in the other (T) the difference could not be exactly determined, but might not have exceeded 2 hours. In all the remaining 48 cases, the stretched muscle survived the loose one. In 17 of these cases, an exact record of the time of death was obtained for one muscle only. These cases, therefore, furnish merely qualitative indication of a positive stretch effect. There remain 31 cases in which a numerical statement of the stretch effect can be made. The stretch effect, given in hours, was (fig. 2):

3 hours	in 6 cases
4-6 hours	in 9 cases
6-8 hours	in 6 cases
8 hours or more	in 10 cases.

TABLE 2

SORT OF MUSCLE	NUMBER OF CASES	AVERAGE STRETCH EFFECT
Sa	6	7.8
T	11	7.9
Fld	5	7.6
G	14	7.1
Se	7	6.4
If	8	4.5
P	2	4.5
Cor	2	3.5

The maximum stretch effect was 30 hours in the case of a pair of *M. gastrocnemii*. Since, however, this enormous difference is a singular occurrence, it may be ascribed to some incidental circumstance. Disregarding this suspiciously favorable case, the average stretch effect calculates to 5.7 hours. Comparing the average stretch effects obtained on different kinds of muscles we find some remarkable differences which, though not yet based on sufficiently large numbers, may be of significance (table 2). The greatest effects were observed on *Mm. sartorii* and *tibiales*, the slightest effects on *Mm. peronei* and *coraco-radiales*. The former group of muscles being much more extensible than the latter, a direct connection between magnitude of the stretch effect and extensibility of the muscle seems to be suggested. In the case of the *Mm. coraco-radiales* and *peronei* the difference is so inconspicuous that the evidence of a true stretch effect could be questioned.

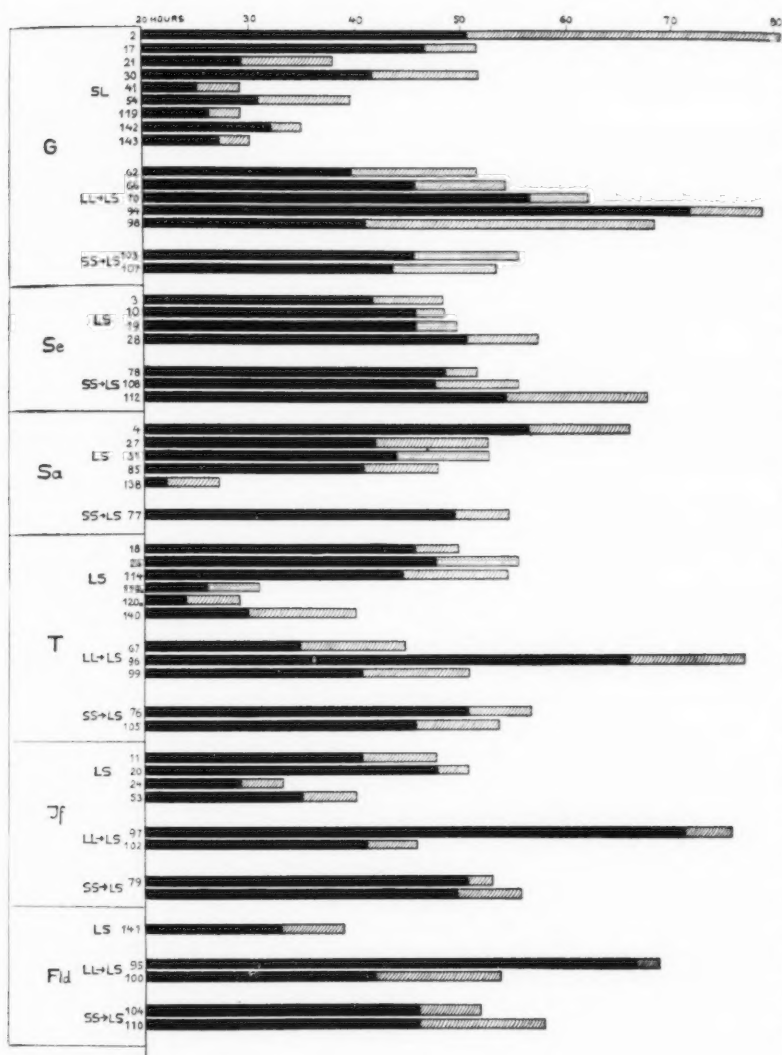


Fig. 2. Graph, representing the results of 53 experiments (the numbers on the left giving the protocol numbers). The total length of each bar represents the survival period of the stretched muscle (minus 20 hours), while the black portion of each bar represents the survival period of the corresponding loose muscle (minus 20 hours). The difference, shown as the cross-hatched part of the bar, represents the positive stretch effect in hours. For abbreviations cf. text.

The first indication of the stretch effect can be noticed as soon as the excitability of the *loose* muscle begins to drop; at this time the threshold of the *stretched* muscle is found to remain almost unchanged. Once excitability has begun to decrease conspicuously, death will occur within a few hours. Comparing the time curves of the decrease of excitability in the loose and stretched muscles (fig. 3), no appreciable difference in steep-

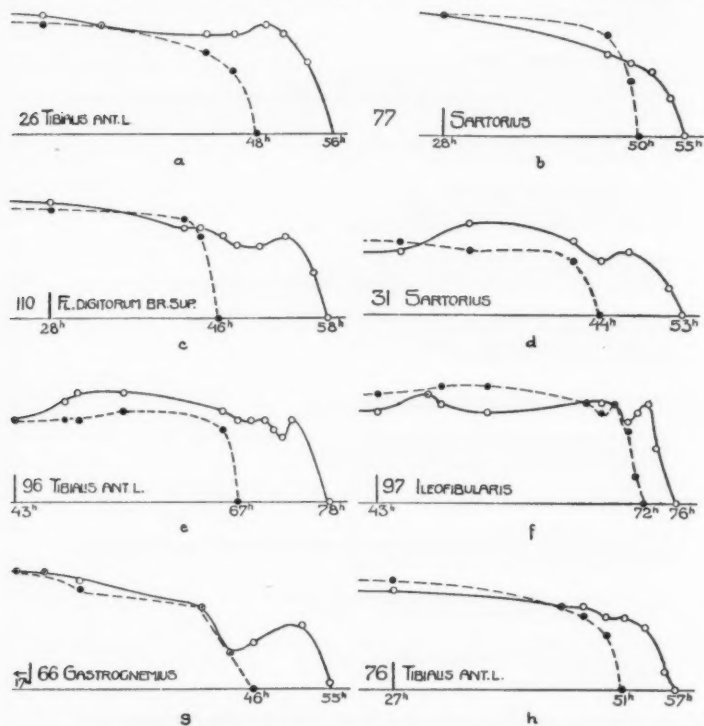


Fig. 3. Graphs, representing the drop of excitability during the latter part of the survival period of 8 pairs of muscles. Abscissae: time in hours after isolation. Ordinates: threshold intensity of stimuli, given in coil distance. Full line: stretched muscle; dotted line: loose muscle. a, d from series LS; b, c, h, from series SS \rightarrow LS; e, f, g, from series LL \rightarrow LS. The vertical mark on the base line in b, c, and h indicates the time of release, in e, f, and g the time of stretch of one muscle.

ness can be observed. Therefore, the stretch effect consists in a delay, for the stretched muscle, of the onset of the decrease of excitability, rather than in a retardation of the rate of decrease. In some cases, a slight increase in excitability was observed immediately before the final decrease started (fig. 3); whether or not this is of significance cannot be said.

C. Series SS. This series of experiments is complementary to series LL. If both corresponding muscles were subjected to approximately equal amounts of stretch, equal stretch effects and, hence, simultaneous death of both muscles could be expected. Only two experiments were recorded. In the first (Sa) the difference in the survival periods of the two muscles was 1 hour; in the second (Se) the difference was $2\frac{1}{2}$ hours. Death, as we see, occurred for both within the same narrow range as in the control series LL.

D. Series LL \rightarrow LS. In this series of 15 experiments, the corresponding muscles after their excision were, at first, both kept loose. Then, after a period of from 11 to 43 hours had elapsed, one of each pair was stretched as in series LS. Again the stretch effect was observed, amounting to (fig. 2):

4-6 hours	in 2 cases
6-8 hours	in 2 cases
8-10 hours	in 1 case
10 hours or more	in 6 cases.

In one case (Fld), the effect was only 2 hours, and for 3 cases the presence of the effect has been ascertained but no numerical statement can be made. The maximum effect was 28 hours in a pair of *Mm. gastrocnemii*. Since in no other case the effect exceeded 12 hours, this one extreme case may again be considered as exceptional. For the remaining cases, the average stretch effect calculates to 8.4 hours. This value, being even somewhat higher than the corresponding value in series LS, proves beyond doubt that the stretch effect is obtained not only if the stretch is applied immediately after isolation, but appears as well if the muscle is subjected to stretch as late as 43 hours after its isolation from the body. Stretch applied 16 hours before the presumptive death of a muscle, as determined by the death of its control (cf. series LL), was still found to be effective, and in one case (T) where stretch was applied 18 hours before the presumptive death, the stretch effect was as much as 10 hours.

E. Series SS \rightarrow LS. This series of 12 cases is complementary to the preceding series D, insofar as both muscles were, at first, kept under identical conditions. Immediately after isolation, each pair of corresponding muscles was subjected to stretch; from this stretch one muscle was released after a period of from 23 to 41 hours. In one case (If) the stretch effect was but slight (2 hours); in the remaining 11 cases, however, a very marked stretch effect was observed, amounting to:

3 hours	in 1 case
4-6 hours	in 1 case
6-8 hours	in 3 cases
8-10 hours	in 2 cases
10 hours or more	in 4 cases.

The average stretch effect was 7.4 hours. The control muscles, i.e., the muscles which later were released from stretch, died between 16 and 24 hours after release. These released muscles, even after 41 hours of previous stretching, were found to behave just as if they had been kept in loose condition from the very beginning of the experiment. Therefore, on the corresponding muscles which were kept under permanent stretch a marked stretch effect was observed.

From the results of series D and E, it becomes clear that in order to prolong the life of the muscle, the maintenance of stretch is required only during the last 16 hours before its presumptive death. It seems to be irrelevant whether, before that period, the muscle was kept loose or stretched. This fact is further corroborated by the following series.

F. Series LS \rightarrow LL. This series includes only 5 experiments. One muscle was subjected to stretch immediately after isolation, the other muscle being permanently kept loose. After a period of 17, 29, or 41 hours, the stretched muscle was released so that henceforth both muscles were under identical (loose) conditions. In one case (G), the survival period of the temporarily stretched muscle was somewhat longer than in the control muscle, but no numerical value was obtained. In another case (G) where the stretched muscle was released after 29 hours (20 hours before the death of the control muscle), a stretch effect of 6 hours was observed. In the 3 remaining cases (G, T, Fld), however, almost no stretch effect was noticeable. In one case there was a difference of 3 hours in favor of the originally stretched muscle and in 2 cases there was no difference at all. This is the more remarkable as the time between the loosening of the stretched muscle and its presumptive death (determined by the death of the control muscle) was only 3 to 9 hours. It is obvious that the experiments of this series are not as consistent as those of the other series. Recalling, however, the unequivocal results of series D and E, one should be inclined to consider as typical the last 3 experiments of the present series, i.e., those where there was no marked stretch effect, while the apparent stretch effect exhibited by the first two experiments would have to be ascribed to accidental circumstances. It may be mentioned that since the muscles of the 3 favorable cases were taken from two different animals, the absence of the stretch effect could not be explained on the basis of an incidental individual refractoriness against stretch. The question remains open until further experimental information is available.

DISCUSSION. The experiments described above prove beyond doubt that isolated muscles which are subjected to maintained stretch retain their excitability and contractility for a longer period than do corresponding muscles under loose condition. The time difference by which the survival period of the stretched muscle exceeds that of the loose muscle is called "positive stretch effect." Since, by control experiments, corresponding

muscles under identical conditions (either both loose or both stretched) were found to die within about 2 hours, a difference of 3 hours or more in favor of the stretched muscle has to be considered as a positive stretch effect. Among the 77 experiments of series C, D and E which were expected to yield a positive stretch effect, only 2 negative results were obtained, showing one a difference of 2 hours and the other of undetermined length in favor of the loose muscle. In all the remaining 75 experiments, the difference was in favor of the stretched muscle (cf. fig. 2). In two extreme cases, the differences were as high as 28 and 30 hours. In general, however, the stretch effect was much less; for 55 cases which can be numerically evaluated (neglecting the two extreme cases), an average stretch effect of 6.6 hours is calculated. The average values of the stretch effect on different sorts of muscles are given in table 2.

Although the number of cases on which table 2 is based is small, it can be considered as certain that the low values obtained with the *Mm. peronei* and *coraco-radiales* are characteristic for these muscles. The apparent unfitness of these two sorts of muscles seems to find an easy explanation in anatomical peculiarities precluding the application of a definite and effective stretch; in the *M. peroneus* extensibility is impaired by the toughness of the adhering fascia, and in the *M. coraco-radialis* by the convergent arrangement of the fibers of this particular muscle.

In this connection, it has to be mentioned that a series of experiments performed three months later (during February) failed entirely to yield a positive stretch effect on any one of the investigated muscles. These latter experiments were done with frogs belonging to the same stock of material as those used in the earlier (November-December) series, but the animals were kept without food during the whole winter; therefore they were in a rather starved condition when the later attempt to repeat the experiments was made. This fact may account for the failure to obtain a positive stretch effect. It is not unlikely that the failure is ultimately caused by an increase in the ratio of connective tissue to contractile substance in the muscle, which would diminish the extensibility of the muscle. In any case, these negative results warn us that not all material is equally suitable for such experiments. This is in full accordance with the experience of Feng in his experiments on the influence of stretch on muscle metabolism (cf. below): He obtained positive results on frogs from England, inconsistent results on frogs from Holland, and negative results on frogs from Hungary, which he explained by his observation that "muscles of Hungarian frogs were considerably and those of Dutch frogs appreciably less extensible than those of English ones."

Turning to the probable factors involved in the stretch effect, one possible assumption can be eliminated at once. That is, that the effect might consist in preventing a contracture from being developed as a result of

transferring the muscle into a slightly inadequate salt solution. This assumption is clearly disproved by the series D and E, where the stretch effect was obtained in spite of both muscles having been kept under identical conditions for more than 40 hours after isolation. The same series seem to furnish convincing proof that the treatment of the muscles during the first part of the survival period is without significance as far as the eventual manifestation of a stretch effect is concerned. Only the condition of the muscle during the last hours of the survival period seems to be decisive. The minimum time before presumptive death when stretch should be applied in order to yield a noticeable stretch effect, has not yet been determined, but this time is, in any case, less than 16 hours.

Stretch may have various effects on the muscle. One particular influence which is responsible for the prolongation of the survival period does not enter into play until shortly before the final drop of excitability. This fact may serve as some clue as to the true nature of the stretch effect. It makes it appear for instance very unlikely that the stretch effect should find its explanation in an underlying effect on metabolic activities. As a matter of fact, the resting metabolism of muscle is very markedly influenced by stretch. Eddy and Downs (3) have shown in 1921 that after stretching a muscle its liberation of carbon dioxide increases to four times the original value. Feng (4) has recently found that in muscles which were subjected to a persistent stretch both heat production and oxygen consumption rose, the latter as much as two- to four-fold. Since his records were taken only over short periods, his findings cannot be immediately compared with our long time experiments. It is left for future investigation to decide whether the increase of metabolic activity under the influence of stretch persists as long as the stretch is maintained or is merely a temporary phenomenon. Neither alternative would offer a simple explanation of our results. If the influence was only a temporary one, with the original increase of metabolic activity gradually vanishing, no difference in the metabolic conditions of the muscles would be expected in series E where both muscles were kept under stretch for a long time. There was, however, a very marked stretch effect obtained in this series. On the other hand, if the influence was persistent, a great difference in the metabolic conditions of the two muscles would be expected in series F, where one muscle was kept stretched for a long time while the other was loose; in this series, however, no considerable stretch effect was obtained. Therefore, it does not seem very logical to assume that there is a direct connection between our stretch effect and some metabolic effects which might be associated with the application of stretch. There is another interesting inference from a comparison of our results with the work of Feng. If there were a permanent increase in metabolic activity under stretch, the total metabolic activity during the survival period must be much greater

in a stretched muscle than in a loose muscle. That means to say that a loose muscle has used up less of the metabolic reserves than has a stretched muscle at the time of death. In that case we would have to conclude that what causes the death of a muscle is not simply metabolic exhaustion.

As a preliminary explanation for the prolongation by stretch of the survival period of a muscle, an hypothesis may be advanced on the following assumptions:

1. Contractility of the muscle fiber is based on the existence of a definitely oriented arrangement of the polar ultramicrosomes of the contractile substance parallel to the axis of the muscle fiber.

2. Death of the muscle fiber consists in an irreversible breakdown of this polar arrangement (disorganization).

3. Stretching the muscle fiber in the direction of its polar axis, by mechanically supporting the polar structure, delays the process of disorganization.

ad 1. It seems to be well established that the elements of the contractile substance are rod-like in shape with a lengthwise arrangement parallel to the axis of the muscle fiber. This is not only morphologically expressed in the longitudinal fibrillar organization of the muscle fiber but is especially evidenced by the phenomenon of bi-refringence which can be observed in every contractile unit, whereby the optical and mechanical axes coincide. As early as 1875, Engelmann (5) advanced the idea of a definite connection between this bi-refringence and contractility of the muscle fiber. This is further corroborated by the fact that the myosin fraction of the muscle which probably contains the contractile substance is double refracting (6).

ad 2. The physiological contraction of a muscle fiber may be associated with a partial breakdown of the polar arrangement of the contractile substance as is emphasized by the decrease of the double refraction during contraction. Under physiological conditions a polar re-arrangement may be supposed to take place after the contraction. In the resting muscle, factors (energy of shape?) may be assumed to be permanently maintaining the polar organization against the natural tendency of its breakdown toward the more stable state of disorientation. As soon as these factors, whatever their nature may be, cease to work the loss of polar organization will become a definitive one. With it must be associated, according to the foregoing assumptions, a definitive loss of contractility.

ad 3. When the internal factors maintaining the polar organization of the contractile substance decrease in activity, external stretch applied in the direction of the polar axis may be able to replace them for a while by offering to the ultramicrosomes tending to disorganize a sort of support counteracting that tendency. Stretch applied to a colloidal substance containing rod-shaped elements results in a polarized arrangement of the previously disoriented particles, the axis of the polarisation coinciding with

the direction of stretch. Therefore, there is good reason to believe that stretch in the direction of an already existing polarisation can also contribute to support such polarisation. This is what we suppose to be the basis of what we have called, in our experiments, the "stretch effect." It is evident that such a conception at once explains why stretch has its effect on the maintenance of contractility only if applied toward the end of the survival period: during the earlier period the *internal* physiological factors mentioned above are sufficiently efficacious to maintain the polar organisation.

SUMMARY

Pairs of corresponding muscles (from the right and left limb) in the frog were isolated and kept *a*, both loose; *b*, one loose, the other permanently stretched; *c*, both stretched; *d*, first both loose, later one of them stretched; *e*, first both stretched, later one of them released; *f*, one loose, the other temporarily stretched. The contractility of the muscles was tested, in intervals, by electric stimulation, and the time when death of the muscles, i.e., loss of excitability and contractility, occurred was determined. The time from isolation until death is called survival period. While the survival periods of different muscles from the same animal differ somewhat and those from different animals differ considerably in length, (from about 30 to 70 hours, fig. 1) the survival periods of corresponding muscles of the same animal were found to be almost equal in length provided both muscles were kept loose (series *a*) or both stretched (series *c*). The maximum difference recorded in such pairs was $2\frac{1}{2}$ hours. When one muscle of a pair was kept loose and the other under stretch (series *b*, *d*, *e*), a marked difference in their survival periods was noted. This difference was in favor of the stretched muscle in 75 out of 77 experiments (fig. 2). This indicates that stretching of these muscles has resulted in prolongation of their survival period. In extreme cases, the prolongation may amount to as much as 30 hours; on an average, the effect is only 6.6 hours (calculated from 55 cases permitting numerical evaluation). The "stretch effect" prolonging the survival period of the muscle is obtained only if the stretch has been acting on the muscle during the *later* stages of the survival period; stretch applied during the *earlier* stages seems to have no effect. As an explanation of the phenomenon the following hypothesis may be outlined: contractility is intimately related with and is dependent on a polar arrangement of the particles of the contractile substance in the direction of the axis of the muscle fiber. With a breakdown of this polar structure contractility is abolished. Such a breakdown will occur as soon as, with approaching death, the factors organizing the polar arrangement cease to work, giving way to the factors with merely disorganizing tendencies. Tension in the direction of the polar axis tends, however, to maintain the polar

structure and, therefore, can replace for some time the normal organizing factors, after these latter have ceased their activities. In this way, by supporting the physical substratum requisite for contracity, stretch has the described effect of prolonging the survival period.

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THE WATER OF THE CEREBROSPINAL FLUID

VARIATIONS OF ITS RATE OF FLOW WITH VARIATION OF VENTRICULAR PRESSURE

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Studies of the chemistry of the cerebrospinal fluid have led to diverse interpretations of the nature of the forces responsible for its separation from the blood. Examination of certain of its constituents has given rise to the conclusion that its formation is dependent upon physico-chemical forces fairly well understood, that the whole fluid is to be regarded as an ultrafiltrate or dialysate of the blood. Analysis of other components has given data which indicate in the opinion of many that forces arising within the cells of the chorioid plexus and ventricular ependyma control the chemical composition of the fluid and that the whole fluid therefore is to be considered a secretion. At the moment it appears probable that no general answer can be returned to this problem but that the behavior of each constituent at the membranes separating blood and cerebrospinal fluid must be separately analyzed and conclusions regarding the nature of the fluid restricted to the particular component under investigation.

The transfer of water across animal membranes, in view of observations from many sources (1, 2, 3), cannot generally be assumed to be due entirely to the forces of hydrostatic pressure and osmosis. In the case of the cerebrospinal fluid, water must pass through capillary walls and then through the epithelial cells of the chorioid plexus and probably a portion of it through the ependymal cells of the ventricles. Can the transfer of this water from the blood to the ventricles be demonstrated to be a function of the effective hydrostatic pressure gradient between capillaries and ventricles? The data to be presented here suggest an answer to this question.

METHODS. The rate of flow of cerebrospinal fluid from the aqueduct of Sylvius has been measured by a method already described in detail (5). After exposure of the fourth ventricle, a small catheter, surrounded by a rubber balloon, was placed into the ventricle and against the aqueduct. The balloon was dilated to secure blockage of the ventricle and the catheter passing through it connected to a calibrated bubble-manometer. A movable fluid-reservoir of large diameter attached to one end of the manometer

permitted determination of the rate of cerebrospinal fluid-escape at any desired intraventricular pressure.

The experiments were all performed on adult cats, kept at normal body temperature under light surgical ether anesthesia. Intracranial arterial pressure was recorded from the peripheral end of the carotid artery. Intracranial venous pressure was measured from the peripheral end of the external jugular vein (5). At the end of the experiments, all animals were promptly killed.

EXPERIMENTAL DATA. In the experiments previously reported (5) on rate of production of cerebrospinal fluid at constant intraventricular

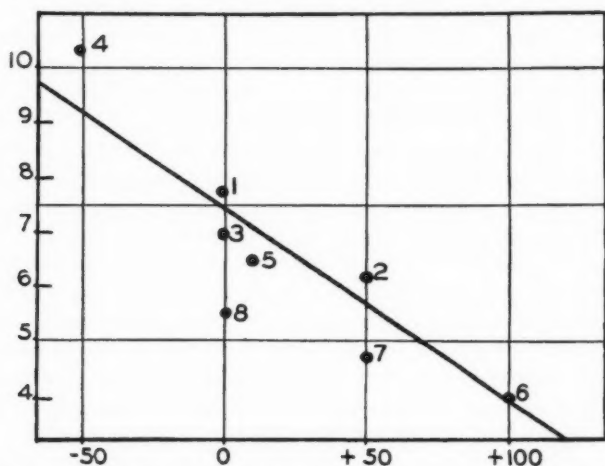


Chart 1 (cat, expt. PCV-11). The abscissae represent intraventricular pressure in millimeters of water. Zero is atmospheric pressure. The ordinates represent units per minute of cerebrospinal fluid flow from the aqueduct of Sylvius; one unit equals 0.002 cc. The points were determined in the order of their numbers. Point 8 indicated a change in the circulation of the choroid plexus and was not used in determination of the line.

pressure, it was noted that the rate of flow was often highly irregular. These periods of irregularity, due to variables incompletely understood, are quite obviously unsuited to an attempt at evaluation of the effect of change of ventricular pressure on the rate of flow. Analysis of the effects of change of ventricular pressure was consequently restricted to those data which satisfied the following tests: First, at a constant intraventricular pressure, the rate of fluid escape must have been a steady one; second, after change of intraventricular pressure, return to the original pressure must have reproduced approximately the original rate. Nine such experiments have been successfully completed.

In chart 1 are presented the results of a typical experiment. Each point was determined by the average rate per minute calculated from a period of six minutes. Readings taken during the first two or three minutes after change of intraventricular pressure were discarded because of possible changes in ventricular volume.

The rate of flow was observed only at intraventricular pressures ranging from plus 110 to minus 50 or minus 100 mm. of water. Higher pressures than plus 110 were not used because too high a pressure was then necessary within the balloon to maintain a water-tight joint between it and the walls of the fourth ventricle. The flow at pressures lower than minus 100 mm. of water could not be investigated due to lack of free communication between the catheter and the third ventricle. This occlusion probably resulted from collapse of the walls of the aqueduct or of rostral portions of the fourth ventricle at low intraventricular pressures.

The eight observations of chart 1 were made over a period of 80 minutes. It will be noted that the average rate near the end of this time (point 8) had changed appreciably from its value at the beginning of the experiment (point 1). This is probably due to changes in effective hydrostatic pressure and rate of flow within the capillaries of the chorioid plexus. All subsequent observations, consequently, were not to be related to those preceding for an evaluation of the effects of altering intraventricular pressure; the animal was in a new condition and further observations necessarily were related to a new base-line of comparison. Because of similar variations, some of the experiments, considered acceptable, have permitted comparison of only five observations and many experiments have had to be completely discarded.

The data of chart 1 show that the rate of flow of cerebrospinal fluid from the aqueduct diminished as ventricular pressure was increased. The observations appear to be distributed about a straight line. This line in many of the experiments was rather inaccurately determined by the data: its intercept on the abscissa corresponding to zero-rate could in some instances be determined only with an error of ± 25 mm. of water.

An effort was made to check these results by measuring the rate of cerebrospinal fluid-flow from a needle or cannula introduced into the cisterna magna and attached to a bubble-manometer connected to a movable fluid-reservoir. It was necessary in these experiments to use pressures in the subarachnoid space so low that the results would not be complicated by variations in the rate of absorption of cerebrospinal fluid into the veins. Measurement of the colloid osmotic pressure of the blood as suggested by Mayrs (8) gave a value of 30 cm. to 32 cm. water. In these experiments, therefore, the rate of escape was measured between pressures of minus 400 to minus 700 mm. water. All of these experiments showed an increase in rate of flow with decrease in pressure in the subarachnoid space. In agree-

ment with the findings of Weed and Flexner (10) it was exceptional to note a significant change in either intracranial venous pressure or the pressure in the Circle of Willis with change in the pressure of the ventricles or subarachnoid spaces.

DISCUSSION. Measurements of the rate of flow of cerebrospinal fluid with change of intraventricular pressure indicated that as ventricular pressure was diminished the rate of flow increased. The observations were distributed about a straight line as has been found by Landis (7) for the capillaries. Similar results followed reduction of the pressure in the subarachnoid space to values below the osmotic pressure of the plasma proteins. To eliminate absorption by the veins of the subarachnoid space, however, it was necessary to work at such low pressures in these last experiments that it is doubtful whether the observations gave an uncomplicated view of the formation of cerebrospinal fluid at the chorioid plexuses. For added to the fluid from this source, there was probably increased flow from the perivascular spaces, transudation from the blood vessels of the subarachnoid space and edema of the brain. These last results are of value, consequently, only in that they offer no contradiction to those obtained from change of ventricular pressure.

As has been noted, ventricular pressure was changed within the range of plus 100 mm. and minus 50 to minus 100 mm. of water. Extrapolation of the line determined by the rates of flow at these pressures to the ordinate corresponding to zero-rate might be expected to give a value from which the pressure in the capillaries of the chorioid plexus could be calculated. There are, however, several incompletely understood factors which make uncertain the meaning of this intercept. It is not known with certainty whether the normal chorioid plexus is permeable to water in the direction from ventricle to blood (4). No final conclusion, consequently, can be made as to whether the intercept is a measure of the lowest effective hydrostatic pressure in the capillaries from which cerebrospinal fluid is being separated or whether it is a measure of mean capillary pressure. Nor has the relationship of the osmotic pressure of the cerebrospinal fluid to the osmotic pressure of the blood been determined with a high accuracy. The conclusion that cerebrospinal fluid is in osmotic equilibrium with blood (6), that the two fluids have the same osmotic pressures (9), is based on measurements of freezing-points. The freezing-point method as used in these determinations has an error so large that data obtained from it cannot safely be used to evaluate the intercept in terms of capillary pressure. An attempt has consequently been made to measure by a direct method the pressure in the capillaries of the chorioid plexus. So much damage must be done to the brain in exposing the plexus of a lateral ventricle, however, and the brain is so unsteady due to arterial and respiratory pulsations, that the effort was entirely unproductive.

The foregoing experiments support the view that the rate of production of the water of the cerebrospinal fluid is a function of the difference between the effective hydrostatic pressure in the capillaries of the chorioid plexus and the hydrostatic pressure in the ventricles. The changes in rate of flow of fluid with change in ventricular pressure are to be explained by assuming a simple process of filtration. A consequence of this explanation is that alteration of the effective hydrostatic pressure in the capillaries of the chorioid plexus will cause changes in the rate of production of cerebrospinal fluid; this point is now being investigated.

SUMMARY

Variations in the rate of production of cerebrospinal fluid with variations of intraventricular pressure have been studied. Within the ventricular pressure-range investigated, the rate of formation of the water of the fluid appears to be a linear function of the difference between the effective hydrostatic pressure in the capillaries of the chorioid plexus and the hydrostatic pressure in the ventricles.

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ON THE QUESTION OF THRESHOLD IN STIMULATION OF THE MOTOR CORTEX

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While engaged in delimiting the anterior boundary of the motor cortex for purposes of frontal lobe ablations in the macaque (*Macacus mulatta*, old terminology, *Macacus rhesus*) one of the writers chanced upon a difference in the strength of induced current necessary to elicit movements from the arm and leg regions. Since threshold values in that particular piece of exploration were a matter of some import it became imperative to know not only the strength of current used, but also the frequency.

After some preliminary experimentation J. A. Myers was able to design and make a device which delivered a current of known frequency (a.c. sine wave of 60 cycles) capable of a variation in strength from 0.1 of a volt to 8 volts. Later, again Mr. Myers was able to furnish frequencies of 7 to 1440 cycles per second at known voltage strength. By use of these two types of stimulation, the writers have explored the cortices of adolescent and of recently born specimens of *Macaca mulatta*, and of the cat. This study then, falls naturally into two divisions,—first, the exploration of the motor cortex with a source of known frequency capable of being delivered in known strengths of current, and secondly, the exploration of the strength of current necessary to reproduce a given movement with different frequencies.²

The indifferent electrode used was the ordinary copper plate wrapped in gauze soaked in N.NaCl solution. The stimulating electrode (unipolar) was a platinum wire mounted on a delicate spring. In the initial experiments, the resistance of the particular animal was not taken; in the later ones however it was taken during the experiment.

In the exploration of the adolescent macaque's motor cortex with the 60 cycle sine wave current certain phenomena became evident. In repeated

¹ E. P. Boynton held a Denison Fellowship during the later stages of this work.

² In the study of the cortices of the young specimens of *Macaca mulatta* Dr. William L. Straus aided us in the analysis of the groups of muscles responding to our stimulations. Both Doctor Straus and Mr. Myers will publish their part of this work independently; for the writers are chiefly concerned with the ability of this cortex to transmit the electric current given it.

stimulation of the same point by decreasing strengths of current not only the phenomena of facilitation, reversal and deviation of response in the sense of Sherrington and Graham Brown appeared but also what might be called a reciprocal of facilitation. Instead of an augmentation or intensification of the muscular response, a diminution and simplification presented itself, a phenomenon which might be named centripetal individuation. Gradual lowering of the strength of the stimulating current may lead not only to centripetal individuation but to deviation as well, to complete deviation or to partial reversal and partial deviation. On the other hand a slight increase in the strength of the current from 1 volt to 2 volts, is able to cause in one stimulation a combination of movements which have appeared separately from threshold-stimulation of points within a small radius, and is in no wise dependent upon the previous stimulation of other cortical points. If, however, the strength of current be increased greatly and suddenly, no movement will ensue.

The lowest thresholds in the motor cortex of these beasts were those for movements of the toes, of the fingers and of the muscles around the mouth, and generally lay toward the central fissure. In every cortex so far examined there were small areas, stimulation of which at liminal voltages resulted in discrete movements of the great toe or of the thumb, of the toes or of the fingers or of the *m. nasolabialis*, uncomplicated by other movements. These are so well located that it is possible to predict the movement resulting from their stimulation providing that the initial stimulus has a current value somewhere in the vicinity of the threshold of the point in question.

In brains which are marked by distinguishing vessels it is possible to stimulate the same small area, after intervals of days, provided of course that the whole procedure is aseptic. In one animal initial stimulation of such a point at 1.6 v. resulted in extension of the wrist (Feb. 28, 1933); fourteen days later (Mar. 14) that same point at 1.6 v. gave extension of the wrist with extension of the fingers, and thirty-five days after the first trial (April 4), at exactly the same voltage, extension of the wrist with flexion of the fingers. On the opposite motor cortex of this same animal a comparable area, stimulated on April 27, resulted in extension of elbow, wrist and fingers at 1.5 v.; on May 9, in the same movement at 1.6 v., and on May 25, at 1.4 v., in flexion at the elbow, extension of the wrist and fingers. On April 27 and May 25, the voltage was lowered to 1.2 with the resulting movement of extension of wrist and fingers, and of extension of fingers respectively. The area is thought to be primary for that movement, not only because the movement of extension of the wrist appears in all these stimulations, but also because of its position in the cortex. It lies more dorsal than the primary finger region. The secondary movement in this case is more susceptible to elicitation at low current values than the

primary. On the other hand if the primary movement be produced from a region of low threshold, centripetal individuation works in the opposite manner, and cuts out the secondary movements.

If the threshold in volts had been determined on the surface (3 v.) and then a protected needle-point electrode be pushed into the cortex so that it lies above the Betz cell-layer the threshold value for the current which duplicates the original movement is remarkably lowered (1 v.). In one particular instance the cortex about a point so used was removed, fixed and serially sectioned. Luckily, the electrode had not pierced the arachnoid, but rather had pushed it ahead so that the usual number of membranes lay between the needle and the fifth layer of the cortex. In this case the lowering of the threshold cannot be explained by the puncture of the membrane. A great deal of the stimulating current must then be dissipated in the upper layers of the cortex.

Again before removal of a region from which movements of the lower arm were elicited, a point was located which gave with stimulation by a current strength of 1.4 v., extension of wrist and fingers. At greater current strengths (2.0 v., 1.8 v. and 1.6 v.) flexion of the elbow, and adduction of the thumb was added. The resistance of the animal, using this point as one of the contacts, was 2,000 ohms. The area was removed, and the total cut surface again explored, but the movements could not be duplicated. The only part of the movement which appeared was extension of the wrist and that at 2.7 v. Raising the current strength to 3.0 v. and total exploration did not uncover a movement similar in its entirety to the first. The resistance of the animal taken with one electrode on the cut surface was 1,500 ohms. Consequently the increase in current strength necessary to activate the cut ends of these corticifugal systems cannot be related directly to the resistance of the animal. Not only does the substance of the cortical layers lower the current necessary to elicit movement in comparison to the stimulation of the cut ends of the fibers beneath it, but it is also able to maintain a certain constancy of co-innervation.

The stimulation of the cortices of the two new-born macaques revealed an interesting difference in maturation. Although approximately of similar birth ages in days, there was a real difference in actual age (gestation age + age since birth) of 21 days. In the younger (161 days, total age) the cortex of the head and face area was sensitive only anterior to the fissura centralis; while in the arm area it was possible to carry positive results in movement forward in three horizontal lines almost to the inferior prefrontal sulcus. The anterior points within each of these lines had a slightly higher threshold than the first point upon the precentral gyrus, just anterior to the central fissura. None of these movements included movements of the fingers; and all were elicited at current strengths of 3 to 6.5 volts. The foot and leg regions were silent even to 8 volts.

In the older new-born (182 days, total age) the sensitive regions were separated into three regions by silent strips, bounded on either side by regions which required appreciable strength of current to elicit movements. Consequently there was no co-innervation present along their adjacent boundaries. These three regions were respectively areas for the head and face, for the arm and for the leg. The movements requiring the least strength of current (3.0 v.) to elicit from this cortex were those of the toes, of the fingers and of the tongue. The sensitive region of the cortex had begun to spread forward in each of these three separate areas. This cortex also showed the phenomenon of centripetal individuation with decrease in the strength of the current used to stimulate a given point, and at times, facilitation as well. On the other hand, increase in the strength of the stimulating current rarely produced facilitation, more often centripetal individuation.

About five weeks after birth (gestation time unknown in this animal) the electrically sensitive region covered almost as much of the cortex anterior of the central fissure as it commonly does in the mantle of the adolescent macaque with the exception that it did not extend proportionately as far forward in the leg region. This motor cortex is however as in the previous brain separated into three regions by two silent strips, each bounded dorsally and ventrally by horizontal lines of high threshold values. Facilitation occurred with increased strength of the stimulating current, centripetal individuation with a series of currents of decreasing strength. In such serial stimulation deviation of response was noted, but not reversal. The movements resulting from stimulation of low voltages were those of the hallux (3.0 v.), those of the fingers (3.0 v.) and those of the tongue and the lips (2.0 v.).

In macaques a year or more of age the total percentage of cortex from which movements can be elicited, approached that characteristic of the adult. The strength of current necessary to gain movement response resembled that of the adult and the muscular groups which responded with the lowest currents were those moving toes, the fingers, the lips and the tongue. Series of stimuli of decreasing strength demonstrated the phenomena of centripetal individuation, reversal and deviation of response; those of increasing strength, facilitation. Co-innervation was a very marked characteristic of points just anterior to central fissure in the arm area, and was present but once on the anterior boundary of the motor cortex.

In the preliminary experiments with various frequencies, cats were used, later two young macaques. In such experiments the cortex was explored first with the 60 cycle sine wave current, in order to determine the threshold of several rather stable points. One of these points was then chosen and explored with different frequencies. The strength of current necessary

to cause the reproduction of the first movement was determined each time before preceding to the use of the next frequency. In any single cat's cortex the same point could be made to yield a similar movement with different frequencies if the strength of current used for stimulus lay in the vicinity of the threshold value for that frequency. And at the end of the experiment (five or six hours later) the strength of current, which was found to be threshold for that frequency, was able to elicit the movement initially produced. The least effective value of the stimulating voltage occurred on either side of 100 cycles, that is, approximately from 80 to 90 to 110 or even 120. At 60 cycles, the frequency used for all the initial explorations, the voltage necessary was often slightly increased over the threshold value for 90. Below 60 cycles the effective voltage rose. In the region of 30 or 40 cycles, the character of the movement shifted, although the muscles involved remained the same. The latent period increased and the length of time consumed in the total contraction was prolonged. At 20 cycles not only was the latent period increased further, but the initiation period was also increased although the contraction when once begun proceeded cleanly. At 10 cycles the latent period was even more prolonged; a general trembling preceded the slow initiation of the movement, after which the contraction took place. At 20 and 10 cycles the strength of current necessary to produce the movement was greatly increased.

Above 120 cycles (in the cat's cortex), the value of the effective voltage might even double itself, until the range 500 to 600 was reached, where the effective voltage again dropped to something like its value for the 100 cycle range. From 600 cycles on, the threshold of the cortical point rose slowly until the vicinity of 1200 cycles was reached; from 1200 to 1400 cycles the strength of current necessary to elicit movement from the more stable points often approached that determined for 10 and 20 cycles. At these higher frequencies the character of the movement was not changed, although the latent period was prolonged. If several cortical points, treated alike to the same frequency were studied together, it was found that some of them often dropped out in the region of 1000 cycles and by no reasonable increase in the strength of current could movement be elicited, although at the end of the experiment the stimulation of these points at previously determined voltage values for a given frequency did not fail to reproduce the initially resulting movement.

Within a very limited range of frequencies, we attempted on the cat to determine whether facilitation and centripetal individuation might not be elicited in a different manner with different frequencies. With a series of increasing strengths of current, facilitation was demonstrated at 60, 90, 120 and 180 cycles; with decreasing strengths of current centripetal individuation at these same frequencies. But at frequencies below 60,

increase or decrease in the strength of stimuli did not produce the facilitation and centripetal individuation characteristic of those frequencies above 60. Increasing the strength of the stimulus even as little as 5 per cent above threshold-value instead of producing a more marked contraction, often resulted in a less marked contraction.

The exploration of the macaque's motor cortex with different frequencies was attempted first in a specimen approximately 1 year old and in a baby 3 days old (179 days, gestation). The first point chosen for the determination of threshold, in the older macaque's motor cortex, yielded with stimulation of 60 cycles and 2.1 volts, flexion of all five digits; the second point, lateral deviation and extension of all toes, including the hallux. The first point was explored with frequencies varying from 60 to 1440 cycles. From 90 to 150 cycles, the strength of current necessary to produce flexion of all five fingers gradually rose from 2.7 v. to 3.5 v. At 175 cycles the threshold for the complete movement was 4.1 v.; at 200, 4.2 v. In interim of cycles from 350 to 550 the threshold value of current strength dropped to 3.8 v., after which it remained in the region of 4.6 and 4.8 v., from 600 to 900 cycles; at 1000, the effective strength of current rose to 5.2 v., to drop back once more in the vicinity of 4.6 and 4.8 for the cycles used between 1100 and 1440. At each of these frequencies, less strength of current produced fractions of the total movement. The first fraction to appear with the lower current strengths was generally flexion of the thumb; as the strength of the stimulating current was raised the index finger was added, then the little finger and last of all, fingers three and four.

The second movement was elicited from this year-old macaque's cortex by frequencies from 7 to 1000 cycles per second, and presented a somewhat similar picture. The minimum value of the effective stimulating voltage was discovered to lie between 90 and 200 cycles. Differences of strength of current as small as 0.1 volt were often sufficient to produce the total movement. In this cortical point there was no lowering of the strength of current in the region of 500 cycles, but similar to the previous analysis, a slight rise of the effective current strength appeared at 1000 cycles. Below 90, the threshold for the movement rose gradually to 2.3 volts at 60 cycles; at 40 cycles the threshold of the movement decreased by 0.1 of a volt. However, at 30 cycles the movement began to change its character, although the actual threshold was but 0.2 of a volt above that which it was at 40 cycles. This change was in the direction of an increase in the time consumed for its maximal contraction. At 20, 10 and 7 cycles the whole movement of lateral deviation and extension of the toes was not duplicated. At 20 cycles the latent period was greatly prolonged and the total movement disintegrated so that another latent period supervened between the two aspects of the movement, deviation and extension. At 10 and 7 cycles trembling only in the short extensors and the interossei were noted, and even then, not in all of them at once.

At the tested frequencies above 82 cycles, either eversion or inversion of the foot, sometimes (particularly well at 90, 120 and 145) flexion of the leg was added with strengths of the stimulating current slightly above the threshold for lateral deviation and extension of the toes. It is clear then that facilitation could be demonstrated in frequencies above 60 cycles and since the determination of threshold values often proceeded from higher to lower voltages, that centripetal individuation was also present. In this brain the working of both facilitation and centripetal individuation was markedly limited, when the stimulating current was 50 cycles or less.

As a contrast to the characteristics of this brain of a year old macaque, the lowest current strengths producing movement in the three day old baby were found in stimulating frequencies between 60 and 90 (3 v.). Above 90 the strength of current necessary to produce the movement of medial rotation of the shoulder rose rapidly, so that at 240 cycles no movement at all was elicited even with 7 volts. Below 50 cycles the movement whose threshold value was 2.5 v. at a 60 cycles frequency did not appear. Therefore the range of frequencies, proving effective for the elicitation of movement in the motor cortex of this baby macaque was narrow. Facilitation and centripetal individuation although present at 60 and 90 cycles were also limited. Although this young cortex was able to transmit to the final common path stimuli given its outer surface, the frequency and the amplitude of the stimuli which can pass through it were circumscribed, probably by the immaturity of its cortical association fibers or of its corticofugal systems.

In the case of the cat's motor cortex, all ranges of frequency used were conducted, in spite of the fact that facilitation and centripetal individuation were limited. Once obtained by stimulation of a given point in the cat's cortex, the movement was not so easily disintegrated into its parts by variation in frequency and in strength of current. Arranged in order of their increasing instability the cat's cortex must be placed between that of the baby macaque and that of the year old rhesus. The influences of the physiological states within the motor cortex which Sherrington regarded as largely the expression of instability making themselves "felt as deviation, reversal of response and facilitation," can be demonstrated to be related to change of the strength and of the frequency of the current used to stimulate a single point. On the other hand, if strength and frequency remain constant and stimulation proceeds in a similar order, the instability of cortical points becomes markedly less. In such studies as these, we are dealing not with the activity of the motor cortex itself, but rather with its ability to handle the type of stimulation given it. And only as that stimulus approximates the nervous impulse as it is found within the animal body can an understanding of the function of this part of the cortical mantle be approached. Certainly, stimulation of the motor

cortex in the manner first described presents a new angle to the characteristics of its behavior.

SUMMARY

By use of graded stimuli and known frequencies, single points within the motor cortex of the cat and of young macaques demonstrated the deviation, reversal of response and facilitation of Sherrington as well as a reciprocal of facilitation, "centripetal individuation." Movements elicited by frequencies of 30 cycles or less generally showed a prolonged latent period and slowing of time to attain the maximal contraction. Stimulation of motor cortex of the year old macaque with frequencies below 20 cycles did not reproduce the movement characteristic of the cortical point in question; rather, fractional parts of the movement appeared. This phenomenon was not noted in the cat's motor cortex, from which movement was elicited at these lower frequencies. Although the strength of current necessary to reproduce any specified movement might be increased above 500 cycles, the movement was not fractional, if the threshold was reached. At these higher frequencies the latent period was increased but the character of the movement did not change.

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THE THEBESIAN VESSELS AS A SOURCE OF NOURISHMENT FOR THE MYOCARDIUM¹

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Accurate clinical analyses and electrocardiographic studies checked by careful post-mortem examinations indicate that in coronary occlusion many of the myocardial changes found at autopsy are due to coronary occlusions occurring months or even years before death (cf. White, 1933). There can be no doubt that the presence of some auxiliary circulation to the myocardium explains the survival of such patients. Whether or not the Thebesian vessels play any part in nourishing the myocardium in these cases is still in dispute. Several other possible types of auxiliary circulation, besides that of the Thebesian vessels, have been demonstrated, viz.:

1. Anastomoses between coronary arteries (Spalteholz, 1924; Gross, 1921; Moore, 1930; Spalteholz and Hochrein, 1931).

2. Extracardiac anastomoses of the coronary arteries with vasa vasorum leading to the venae cavae, the aorta, the pulmonary vessels, and the intervascular reflections of the parietal pericardium (Langer, 1880; Hudson, Moritz and Wearn, 1932). Redwitz (1909), Woodruff (1926), and Robertson (1930) have also shown the presence of extracardiac anastomoses. Thorel (1903) and Moritz, Hudson and Orgain (1932) have found the pericardial anastomoses especially plentiful in adhesive pericarditis.

3. An "ebb and flow" in the coronary sinus and its branches leading to a cyclic reversal of flow with each heart beat (Batson and Bellet, 1930).

A number of cases with double coronary occlusions have been reported which, according to the clinical and pathological analyses, must have been present for some time before death (Pratt, 1898; Thorel, 1903; Leary and Wearn, 1930; Cabot and Mallory, 1930; Scott, 1929, and others). Anastomoses between coronary arteries can be of no avail if both coronary arteries are occluded: hence each of the other auxiliary circulation pathways has been suggested at some time as a source of nourishment. The

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possibility that the Thebesian vessels could be the pathway for blood supplying the myocardium was suggested first by Pratt (1898) and more recently by Kretz (1927) and Wearn (1928).

The demonstration of anatomical connections between the Thebesian vessels and the intramural coronary circulation was first made in 1706 by Vieussens and independently in 1708 by Thebesius. This has been confirmed by many workers, notably Langer (1880) and more recently Gross (1921), Wearn (1928), and Grant and Viko (1929). The evidence seems clear that the Thebesian vessels are remnants of the extensive sinusoidal blood supply coming off from the heart chambers in the embryo (cf. Lewis, 1904; Grant, 1926; Bellet and Gonley, 1932), and that they are to be found in the adult hearts of fishes, reptiles and mammals (Grant and Regnier, 1926). There is no doubt that the Thebesians connect with the intramural coronary circulation either *a*, through the coronary veins and venules, or *b*, through the capillaries and arterioles (Wearn, 1928; Grant and Viko, 1929; Bellet, Gonley and McMillan, 1933); but Wearn's contention (1928) that the Thebesians join coronary arteries directly has been questioned by Grant and Viko (1929).

There is direct evidence that these Thebesian vessels act as auxiliary veins. The work of Morawitz and Zahn (1912), of Markwalder and Starling (1913), and of Anrep and Häusler (1929) shows that about 40 per cent of the blood from the coronary arteries of the living heart enters the heart cavities by way of the Thebesian communications. Studies of dead hearts obtained at autopsy show that, as a rule, most of the fluid—60 to 90 per cent (Wearn and Zschiesche, 1928), 80 per cent (Crainicianu, 1922; Kretz, 1927)—enters the heart via the Thebesian vessels. In some preparations, however, Wearn and Zschiesche (1928) found the reverse.

The case recently reported by Bellet, Gonley and McMillan (1933) of a heart in a sixteen-year-old boy, where the large coronary arteries and veins were almost completely destroyed by a tuberculous myocarditis and where there were extensive connections between the intramural veins and the heart cavities by dilated Thebesian vessels or sinusoids, favors the idea of the Thebesian auxiliary route. Pratt (1898) demonstrated that the isolated heart of a cat could be nourished from the heart chambers and this has been confirmed by Wearn (1928) and others. The conclusion that the flow occurs via the Thebesian vessels which was made by these authors can be objected to on the ground that the possibility of a cyclic flow in the coronary sinus, or a flow via the extracardiac anastomoses was not excluded. Stella (1931) was unable to demonstrate any back flow into the coronary arteries of the living dog from the heart chambers on cutting the coronary arteries, and in a later study (1932) he found that Chicago blue injected into the left ventricle after the coronary arteries were tied did not penetrate the heart for more than a millimeter or two and presum-

ably did not enter the intramural coronary vessels. Unfortunately Stella reported no histological examinations so that his evidence is incomplete. His experiments may be objected to further, on the ground that his pro-

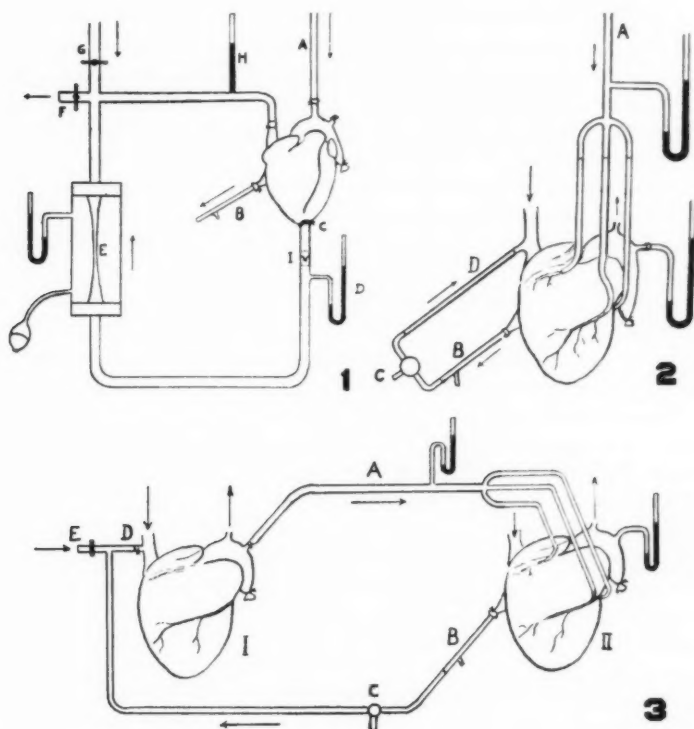


Fig. 1. Modified heart-lung preparation used in preliminary experiments. A, coronary perfusion tube from reservoir; B, Morawitz cannula; C, "arterial" cannula; D, arterial pressure manometer; E, peripheral resistance; F, bleeder tube; G, infusion tube; H, venous pressure manometer; I, valve.

Fig. 2. Perfusion set-up with direct cannulation. A, coronary perfusion tube from reservoir; B, Morawitz cannula; C, three-way stopcock; D, return tube for coronary sinus blood.

Fig. 3. Cross-circulation set-up with direct cannulation. A, coronary perfusion tube from donor dog; B, Morawitz cannula; C, three-way stopcock; D, return tube for coronary sinus blood; E, infusion tube.

cedure of interrupting the coronary flow suddenly would lead to such rapid depression of the heart's action that the time was insufficient for establishing or even demonstrating the presence of physiologically effective auxil-

iliary pathways, such as the Thebesian vessels. It is well known clinically that the time factor in the development of a coronary occlusion is an important, if not the most important, factor in determining the establishment of an adequate auxiliary circulation and the survival of the patient.

We felt that further investigation of the rôle of the Thebesian vessels as a source of nourishment for the myocardium was desirable. Therefore, we carried out a series of experiments on living hearts in dogs, in which precautions were taken to avoid sudden interference with the coronary flow at the time of the experiment, and, in addition, to exclude auxiliary circuits other than the Thebesian vessels. We attempted to provide experimental conditions in which the coronary circulation was isolated from the systemic circuit, so that any substance injected into the systemic circuit could find its way into the coronary vessels only through the Thebesian communications.

PROCEDURE AND RESULTS: PRELIMINARY EXPERIMENTS. *Method.* The preliminary experiments were made with a modified heart-lung preparation. The arrangement is shown in figure 1. A heart-lung preparation was arranged with the usual type of peripheral resistance, *E*, and with the venous return cannula in the superior vena cava. The "arterial" cannula, *C*, was placed directly into the cavity of the left ventricle through the wall in the region of the apex instead of in the aorta; back flow into the ventricle was prevented by a valve, *I*, in the circuit. The coronary arteries were perfused by the Langendorf method through the innominate artery with defibrinated blood at a pressure of 100 mm. Hg (except in expt. C18), and the blood was drained from the coronary sinus by a Morawitz cannula, *B*. The perfusion pressure was supplied by gravity, and the blood was returned to the perfusion reservoir and oxygenated at the same time by compressed air. By means of bleeder, *F*, and infusion, *G*, tubes in the venous return tube, the total blood volume of the systemic circuit could be regulated. The whole apparatus was enclosed in an electrically heated cabinet controlled by a thermostat maintaining the blood temperature constant at 39°C.

Dogs anesthetized with morphine and Na barbital were used. After the chest was opened, the blood supply to and from the head and forelimbs was tied off and the cannula for coronary perfusion inserted into the innominate artery. At this point sufficient heparin was injected to prevent the animal's own blood from clotting. The superior vena cava cannula was next inserted, and then the cannula into the left ventricle. Finally the descending portion of the aorta, the azygos vein, and the inferior vena cava were tied off, and the Morawitz cannula was inserted into the coronary sinus.

The peripheral resistance and the blood volume of the systemic circuit were adjusted so that the mean arterial pressure varied from 8 to 60 mm.

Hg in the various experiments (see table 1). Then the bleeder and infusion tubes in the venous return tube were sealed, disconnecting the coronary

TABLE 1
Thebesian circulation experiments with modified Langendorf method

NUMBER OF EXPERIMENT	PRESSURE		MATERIAL INJECTED	FINDINGS IN CORONARY SINUS BLOOD	FINDINGS IN HISTOLOGICAL SECTIONS OF HEART	REMARKS
	Coronary artery	Left ventricle				
	<i>mm.Hg</i>	<i>mm.Hg</i>		<i>Bismuth</i>		
C7	100	56	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	++++	Bismuth (?) in arteries and veins	
C8	100	48	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	++	Bismuth (?) in veins	
C10	100	38	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	Negative	Negative	Large amount of hemorrhage into tissues
C11	100	60	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	+	Bismuth in large amounts in endocardial pockets and sinusoidal spaces	
C14	100	10	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	++++	Negative	Hemorrhage into tissues

TABLE 1—*Concluded*

NUMBER OF EXPERIMENT	PRESSURE		MATERIAL INJECTED	FINDINGS IN CORONARY SINUS BLOOD	FINDINGS IN HISTOLOGICAL SECTIONS OF HEART	REMARKS
	Coronary artery	Left ventricle				
	<i>mm.Hg</i>	<i>mm.Hg</i>		<i>Bismuth</i>		
C15	100	50	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	+	Negative	Hemorrhage into tissues
C18	68-64	55-20	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	Negative	Bismuth in sinusoidal spaces	+ bismuth in systemic blood
Controls						
C6	100	?	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	++++	Bismuth in veins and some arteries	Bismuth found in apparatus; two circuits not isolated
C12	100	8	50 cc. of a 10 per cent suspension of bismuth oxychloride into superior vena cava	++	Bismuth in endocardial pockets and sinusoidal spaces, and in large veins and arteries	Sinus cannula in left ventricle
C9	100	44	Bismuth injected into coronary inflow tube	Negative	Large amounts of bismuth in small arteries, veins, and capillaries	++ bismuth in systemic blood

circuit from the systemic. At this point 50 cc. of a 10 per cent suspension of bismuth oxychloride in 0.9 per cent sodium chloride solution were injected into the superior vena cava, and the heart permitted to beat for another 10 minutes.

The blood from the coronary sinus was collected and analyzed chemically for bismuth. The heart was fixed and stained to permit histological examination for bismuth. The fit of the Morawitz cannula in the coronary sinus was verified at autopsy.

The analysis of the blood for bismuth was briefly as follows:

All organic material was oxidized by nitric and sulphuric acids and superoxol. The bismuth was precipitated as the oxalate, and then dissolved in sulphuric acid, forming bismuth sulphate and oxalic acid. The amount of oxalic acid present was then determined by titration with potassium permanganate, and from this the amount of bismuth which was in combination with it was determined. In the tables of results, tables 1 and 2, bismuth over 100 mgm. per cent is designated by + + + +. Lesser amounts are designated accordingly, + denoting anything from a trace to 5 mgm. per cent.

In the histological sections, the bismuth was precipitated as the sulphide by treating the blocks with an aqueous solution of hydrogen sulphide overnight at 56°C. The bismuth thus appears black against the red eosin stain of the heart muscle. When bismuth was present in large amounts, as in the endocardial pockets, it showed up clearly in sections as a dense black mass. It was not easy to distinguish small particles of bismuth with certainty from a dark brown precipitate sometimes resulting from the staining process.

Results. Results, summarized in table 1, show that bismuth appeared in the blood issuing from the coronary sinus in varying amounts in five of the seven experiments. The absence of bismuth in the other two experiments is not surprising in view of a similar negative finding in one of the three controls. The controls were of three sorts: 1, where the systemic circuit was in direct communication with the coronary circuit by means of the "bleeder" tube in the vena cava which was permitted to empty into the coronary inflow reservoir (C6); 2, where the Morawitz cannula was found to have entered the left ventricle (C12), and 3, where the bismuth was injected into the coronary inflow tube (C9). Chemical examination for bismuth in the systemic blood was made twice (C9 and C18) and in each case its presence demonstrated.

Histological examination showed bismuth in large amounts in the endocardial pockets and sinusoidal spaces (fig. 6). Sometimes the pathway could be followed from the Thebesian foramina into the sinusoidal spaces leading into the myocardium (fig. 5). Many particles of bismuth which were obviously too large to pass into the capillaries and small vessels of the myocardium were deposited in these sinusoidal spaces. In two of the seven experiments (C7 and C8) finely divided particles of a dark material were present in considerable amounts in the smaller intramural vessels. The amount of dark material in the small vessels in these sections was con-

siderably larger than could be demonstrated in a series of hearts stained in the same way but in which no bismuth was injected. However, the appearance of the dark material was similar to that seen in small vessels in experiments where bismuth was injected directly into, or allowed to enter, the coronary circuit (C6, C9 and C12). In the five other experiments bismuth could not be identified with certainty in the coronary vessels.

Critique of preliminary experiments. These preliminary experiments suggest that blood can enter the coronary system via the Thebesian vessels even when the pressure in the coronary arteries is higher than in the heart cavities. However, there are serious objections to the method. The bismuth might have entered the coronary circuit from the left ventricle via the aorta. It is not impossible that during systole the pressure in the left ventricle might have risen above the coronary perfusion pressure, opened the semilunar valves, and forced blood into the aorta, especially when the difference in the mean pressures was around 30 to 40 mm. Hg (as in expts. C7, C11 and C18). Against this objection is the fact that bismuth appeared in the blood from the coronary sinus without any appearing in the large superficial arteries as might have been expected if the portal of entry was the aorta. When bismuth was injected into the coronary infusion tube, it was found in large amounts in the large superficial coronary arteries (expt. C9). It was decided, nevertheless, in view of this possible objection, to modify our technique in the final experiments.

FINAL EXPERIMENTS. *Method.* The essential modification in these experiments was the direct cannulation of the three main trunks of the coronary arteries, the main trunk of the right, and the descending and circumflex trunks of the left. A stretch of 1 cm. of each of these vessels was dissected free, and a cannula inserted in each about 1.5 cm. from the mouth, and the central end tied off. The cannulated coronary arteries were perfused either directly from a reservoir of defibrinated blood at a pressure of 60 to 100 mm. Hg (expts. C16, C17, C19 and C26, table 2), or, in the cross-circulation experiment (C24), from the left subclavian artery of the donor animal. The arrangements for these preparations are shown in figures 2 and 3.

Fig. 4. Bismuth (black particles) which has penetrated almost to the epicardial surface in the region of superficial branches of a coronary artery and vein. *Ep.* shows epicardial surface of heart. Magnification $\times 15$ (reduced $\frac{1}{2}$).

Fig. 5. Bismuth (black particles) filling the sinusoidal spaces within the heart wall. Magnification $\times 20$ (reduced $\frac{3}{4}$).

Fig. 6. Thebesian orifice filled with bismuth. Magnification $\times 25$ (reduced $\frac{1}{2}$).

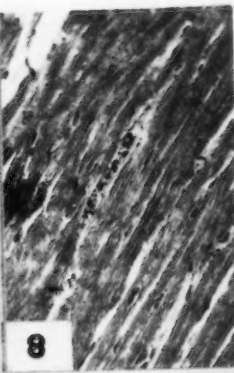
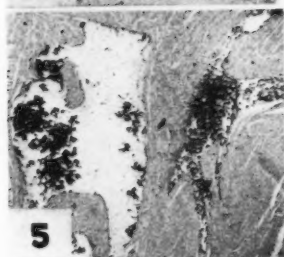
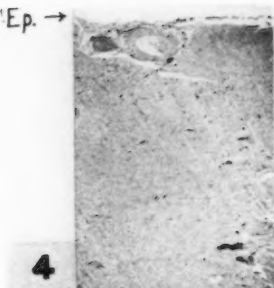
Fig. 7. Small artery containing cocci. Magnification $\times 750$ (reduced $\frac{1}{2}$).

Fig. 8. Small vessel containing cocci. Magnification $\times 500$ (reduced $\frac{1}{2}$).

Fig. 9. Small vessel deep in the heart muscle filled with cocci. Magnification $\times 750$ (reduced $\frac{1}{2}$).

Fig. 10. Capillary within the heart muscle filled with cocci. Magnification $\times 1600$ (reduced $\frac{1}{2}$).

Ep. →



Figs. 4-10

TABLE 2
Thebesian circulation experiments by direct cannulation of coronary arteries

NUMBER OF EXPERIMENT	CORONARY ARTERIES SUPPLIED BY	PRESSURE		MATERIAL INJECTED INTO SUPERIOR VENA CAVA	FINDINGS IN CORONARY SINUS BLOOD	FINDINGS IN HISTOLOGICAL SECTIONS OF HEART	REMARKS
		Coronary artery	Left ventricle				
		<i>mm. Hg</i>	<i>mm. Hg</i>				
C16	Perfusion	100	?	50 cc. of a 10 per cent suspension of bismuth oxy-chloride	+ Bismuth	Bismuth in sinusoidal spaces	
C17	Perfusion	100	10	50 cc. of a 10 per cent suspension of bismuth oxy-chloride	No Bismuth	Bismuth in sinusoidal spaces; bismuth (?) in arteries, veins, and capillaries	
C19	Perfusion	100	50-60	50 cc. of a 10 per cent suspension of bismuth oxy-chloride	+ Bismuth	Bismuth (?) in arteries and small veins	No bismuth in control of coronary sinus blood run before bismuth injection. Systemic blood showed + bismuth after injection
C24	Cross circulation	?	?	100 cc. of a pure culture of killed hay bacilli	Hay bacilli found in blood smears	Hay bacilli found in sinusoidal spaces, small arteries, veins, and capillaries	

TABLE 2—Concluded

NUMBER OF EXPERIMENT	CORONARY ARTERIES SUPPLIED BY	PRESSURE		MATERIAL INJECTED INTO SUPERIOR VENA CAVA	FINDINGS IN CORONARY SINUS BLOOD	FINDINGS IN HISTIOLOGICAL SECTIONS OF HEART	REMARKS
		Coro-nary artery	Left ventri-cle				
		mm.Hg	mm.Hg				
C26	Perfusion	60	60-100	800 cc. of a thick suspension of micro-cocci (Staph. albus)	A few cocci found in smears	Cocci found in large artery, vein, small arteries and veins, and capillaries	

Heparin was used as an anticoagulant. A Morawitz cannula, *B*, was placed in the coronary sinus, the fit being confirmed post-mortem in each experiment. The blood was returned to the superior vena cava of the same dog in the infusion experiments or to the donor dog in the cross-circulation experiment (tube *D*, figs. 2 and 3), until the foreign material was injected. Then the blood was diverted into a collecting flask by means of a three-way glass stopcock, *C*.

In both systems a heart-lung-head preparation was made, the head circuit acting as a peripheral resistance for the left ventricle. The blood supply to the body and limbs was blocked. By using the Morawitz cannula as a bleeder tube and by inserting a tube, *E*, connected with a reservoir in the superior vena cava of the donor animal (in the cross-circulation experiment), the volume of the preparation could be adjusted at will. It was found that the defibrinated blood used in the reservoir had to be filtered four or five times and then passed through the lungs of an animal before being ready for use.

By cannulating the coronary arteries, the isolation of the coronary circuit from the systemic circuit was assured, except for the Thebesian connections. If in these preparations foreign material injected into the superior vena cava appeared in the coronary sinus blood or in the coronary vessels, the deduction must follow that it entered the coronary circuit via the Thebesians. Only a few crucial experiments were performed because of the technical difficulties of making the preparation.

Results of bismuth experiments. The results are summarized in table 2. Bismuth was found in the blood from the coronary sinus in two out of three experiments, although the amounts were smaller than in the preliminary experiments. In experiment C19 the concentration of bismuth in the systemic circuit was also determined and found to be of the same order as in the coronary circuit of this experiment. These experiments show that

blood can pass from the heart cavities into the coronary circuit in the living "normal" heart even when the pressure in the coronary arteries is considerably higher than in the left ventricle.

Bismuth was found histologically in sinusoidal spaces in two of the three experiments, and material, presumably bismuth, was found in the intramural coronary veins, arteries and smaller vessels. In one of these experiments (expt. C17) the bismuth appeared in histological sections of the intramural coronary system even though absent in the coronary sinus blood. Taking the evidence from both chemical analysis of the sinus blood and histological sections of the myocardium, bismuth passed from the heart cavities into the coronary circuit in every experiment.

Critique of bismuth experiments. Bismuth was used in all these experiments since it is an inert substance and must necessarily be conveyed through already patent passageways. The objections to bismuth are its weight and viscosity. This probably prevents its passing into the coronary vessels as readily as would blood itself or a vital dye. Dyes were not used because of the possibility that they might pass into the coronary circuit by absorption through intact vessel walls. Bismuth had another disadvantage—it tended to settle in and block up the lung vessels, so that the right heart often dilated. The variability in the amount of bismuth found in the sinus blood can be accounted for in part at least by a variable filtering out process in the lungs.

Bismuth could not always be identified with certainty in the histological sections. When present in large amounts, as in the endocardial pockets, it showed up clearly in sections as a dense, black mass. In the coronary vessels, however, it was difficult to distinguish fine particles of bismuth from a dark brown precipitate which sometimes appeared even in uninjected hearts as a result of the staining process. While we felt we could distinguish bismuth even in small amounts, this was based on personal opinion.

Results of bacteria experiments. In the last two experiments, therefore, heavy suspensions of killed bacteria suspended in normal saline solution were used for injection material. This provided a more fluid material which was less apt to embarrass the heart, and which could be positively identified in histological sections. These last experiments were terminated and the hearts were fixed in formalin within fifteen minutes after the injection.

In experiment C24, 100 cc. of a suspension of a pure culture of killed hay bacilli were used; in experiment C26, 800 cc. of a suspension of a pure culture of killed staphylococcus albus were used.

Smears of the blood from the coronary sinus showed a few hay bacilli in experiment C24, and a few cocci in experiment C26.

In the histological sections of experiment C24 large numbers of hay

bacilli were seen almost completely filling many of the sinusoidal spaces in some sections. Unmistakable small intramural arteries, venules and capillaries were filled with deeply stained bacilli of the same type as was injected. None was identified in larger coronary vessels. In many fields one small capillary or artery was often completely filled with bacteria while the surrounding vessels in this same field were entirely free of bacteria. Several filled capillaries, small arteries, or spaces might appear in one field, but usually the proportion of filled vessels was small.

In experiment C26, examination of the sections showed cocci of the type injected in small intramural arteries, venules and capillaries, as well as in the smaller superficial branches of the coronary arteries and veins (see figs. 7, 8, 9 and 10). The identity of the arteries in which bacteria were found was checked by an elastic stain. The larger superficial vessels showed comparatively smaller numbers of cocci than some of the small deep vessels. As in experiment C24, the capillaries and small arteries showing many bacteria were usually found along with others showing none at all. The total number of injected vessels in any field was always small. In a few cases it was possible to trace a small artery through several consecutive sections. There is clearcut evidence in experiments C24 and C26 that bacteria passed into the coronary circuit.

Critique of bacteria experiments. The use of bacterial injections was less satisfactory than the bismuth injections as a means of identifying the amount of foreign material actually reaching the coronary sinus, since the number of bacteria carried over was necessarily small and the total amount of blood collected from the coronary sinus was 500 cc. or more. Stained smears, therefore, might or might not chance to show bacteria, and only after careful and extensive search were a few bacteria of the type injected identified in smears made from the coronary sinus blood in experiments C24 and C26. No quantitative estimation of the number of bacteria carried over was attempted. Histological sections of the heart muscle proved much more satisfactory. Bacteria could be clearly identified in the intramural vessels.

Objection may be made that bacteria could have been present in the blood used for perfusion, but in such a case the distribution of bacteria-filled vessels would have been much more general and the greater number of bacteria would have appeared in the superficial coronary arteries rather than in the sinusoidal spaces and small, deep-lying vessels. In our experiments only a few fields showed bacteria in vessels, and in these fields the bacteria were present in only a few of the vessels. It would also be carrying coincidence too far to assume that the contamination in each case was the same as the killed cultures used, hay bacilli in experiment C24, and staphylococci in C26. Previous infection of the heart muscle itself seems highly improbable. Such an infection would not have re-

sulted in filling the lumina of blood vessels with one type of bacteria. There would have been some perivascular infiltration or an increase in the cellular elements of the blood itself. A large series of uninjected dog's hearts were examined histologically and in none did we observe any bacteria in the intramural blood vessels. The possibility of migration of bacteria through the intact vessel walls was avoided by using killed cultures.

DISCUSSION AND THEORETICAL CONSIDERATIONS. The experiments here presented show that the Thebesian channels, which have been shown to act as exits for the coronary circulation, can also act as entrances in the "normal" beating heart. The preparation in our final experiments was such that foreign material, bismuth and bacteria, appearing in the isolated coronary circuit following introduction in the systemic circuit, could do so by passing through Thebesian channels. Hence, there appears to be a sort of ebb and flow in the Thebesian vessels.

Objections may be raised that these experiments did not exclude all the other auxiliary pathways, since 1, the coronary veins other than the coronary sinus were patent, and 2, some of the extracardiac anastomoses with the large vessels of the heart might still have been intact. It is conceivable that the material might have entered *only* by these pathways. Against this unlikely possibility the following arguments may be brought.

1. The ebb and flow which might occur in the patent coronary veins—the coronary sinus having been excluded—is relatively small compared with that occurring in the Thebesian channels. If an ebb and flow can occur in the small coronary veins it must, as will be shown below, also occur in the Thebesian channels, particularly since it is easy to show large amounts of bismuth and bacteria in the sinusoids almost as far as the epicardium (cf. fig. 4).

2. The pressure relations between the large blood vessels and the coronary arteries except in experiment C26 were such that the pressure was definitely higher in the coronary arteries than in the other vessels. Consequently the material, if it entered the coronary circuit from these channels via extracardiac anastomoses must have done so against pressure, which is highly improbable. Further, the anastomoses with the pericardium and mediastinum, which are the most numerous, were eliminated by removal of the pericardial sac. The anastomoses with the aorta and pulmonary artery were eliminated by dissecting free and isolating the upper 1 cm. of the three main trunks of the coronary arteries. The presence of anomalous accessory coronary arteries was excluded by careful examination of the sinuses of Valsalva post-mortem.

The experiments fail to quantitate the amount of blood that can enter the intramural coronary circuit in the normal beating heart via the Thebesian vessels, although they would tend to show that the amount is not

large. Nor do the experiments show whether or not the Thebesian vessels connect directly only with veins, venules, arterioles and capillaries, or with arteries as well. As long as the Thebesian channels can be shown to act as a source of blood supply for the coronary capillaries, it matters little physiologically where the communication is made.

Two problems merit further consideration: 1, the mechanism by which the blood enters the coronary arteries from the cavities of the heart, and 2, the importance of the Thebesian channels as an auxiliary circuit when one or both coronary arteries are occluded.

Kretz (1927) appears to be the only one who has definitely stated an opinion as to the mechanism of inflow via the Thebesian vessels. Most authors have been unable to account for such a flow. Kretz stated that inflow occurs during systole when the intraventricular pressure is greatest and the intertrabecular spaces more accessible. During diastole he believed that the flow is reversed. His explanation may be objected to on the ground that it has not been shown that the intertrabecular spaces are more accessible during systole; in fact, the opposite is generally accepted as the case. Furthermore, while the pressure within the heart cavities is greatest during systole, the pressure within the heart wall is also greatest during this phase.

In attempting to explain the mechanism by which blood enters the coronary circuit via the Thebesian vessels, the various parts of the coronary circuit should be considered separately. In fact, we are of the opinion that such a division would do a great deal in clarifying the controversy concerning the dynamics of coronary flow in general which has arisen from the work of Anrep (1926), Anrep and Häusler (1929), and Rössler and Pascual (1932) on the one hand, and that of Hochrein (1932) and Rein (1931) on the other.

The coronary vessels can be divided into *a*, superficial arteries and veins, and *b*, intramural vessels of 1, the left ventricle; 2, the right ventricle; 3, the intraventricular septum, and 4, the auricles. Just as it is now clear that the flow in the superficial arteries is different from that in the superficial veins both in amount and in cyclic variation, so it is likely that the flow is different in each of the four categories of intramural coronary vessels. There can be no question from recent work that the musculature of each heart chamber acts in two ways. At the onset of contraction it "milks" blood out of the intramural vessels into the superficial coronary veins and heart cavities (Anrep, 1926)—and to a lesser extent into the superficial coronary arteries (Cotton and Wiggers, 1933). During the remainder of contraction the heart muscle acts as a dam preventing the ingress of blood into the intramural vessels (Anrep, 1926).

The fact, however, has been apparently overlooked, that the "milking" and especially the damming up processes are not equally effective in all parts

of the intramural circuit. The systolic flow through the intramural coronary vessels is different, therefore, in the various chamber walls. In this regard, the left ventricle should be separated from the other chambers. Since the left ventricle is the most powerful of all the heart pumps, one would expect that these processes of "milking" and damming would be greatest here. By the very nature of things one would expect that during ventricular systole the blood flow would be stopped in the intramural vessels of the left ventricle since it is this ventricle which is creating the driving force, the aortic pressure, which gives the pressure head for the coronary flow. In the auricles and in the right ventricle there need be no cessation of flow during the contraction of these chambers since the compressing force created by these chambers is relatively small when compared with the aortic blood pressure developed at the same time.

Practically no attention has been given to the possibility that during relaxation (which includes the isometric relaxation and rapid filling phases) conditions are ideal for a flow in the intramural vessels, the reverse of that during the onset of contraction. At the onset of relaxation the pressure around the intramural vessels will be less than in the heart cavities and in the superficial coronary veins so that a reflux of blood could occur. In fact, the same sort of "suction action" can be exerted in this regard as occurs in the filling of the heart (cf. Katz, 1930). True, there will be a considerable inflow from the superficial coronary arteries, but this will not necessarily prevent the entry of blood into the intramural vessels from the superficial coronary veins and from the heart cavities. This line of reasoning has already been applied to the coronary sinus by Batson and Bellet (1930) and it holds equally well for the Thebesian channels. It is during this period of the cardiac cycle, we imagine, that the materials in our experiments pass from the heart cavities through the Thebesian vessels.

When the coronary arteries are occluded the filling of the intramural vessels via the Thebesian vessels (and superficial coronary veins) will be enhanced for several reasons: 1. The competition with the arterial inflow will be lessened or abolished depending on whether the coronary arteries are partially or completely occluded. 2. The Thebesian channels increase in number and cross section area depending on the time taken for the coronary occlusion to develop (cf. Case Report of Bellet, Gonley and McMillan, 1933). 3. The blood flow during diastasis (the period when the heart is relaxed) would tend to be the reverse of normal. Blood flows normally in this period from the coronary capillaries toward the heart cavities (and superficial coronary veins) because the capillary pressure is higher than that within the heart chambers (and the veins). In severe coronary occlusion the pressure gradient would tend to be reversed during this phase, depending on the amount of obstruction interposed between the coronary arteries and capillaries. Experiments are in progress in this laboratory to test out these speculations.

While we do not desire to minimize the rôle of other auxiliary circulations, our experiments suggest that the Thebesian channels are also of importance in maintaining the nourishment of the heart when coronary arteries are occluded.

SUMMARY AND CONCLUSION

1. Bismuth was conveyed from the heart chambers into the coronary sinus blood in the beating heart with the coronary circulation isolated from the systemic, in seven out of ten experiments. In two of these experiments the possibility of any connection between the systemic and the coronary blood by means of extra cardiac anastomoses was excluded by perfusing directly into the coronary arteries.

2. In preparations of beating hearts with a completely isolated coronary circulation a suspension of a pure culture of killed bacteria was injected into the superior vena cava. The same bacteria could be demonstrated in the sinusoidal spaces, the capillaries, the small intramural arteries and veins, the superficial branches of the coronary arteries and veins, and also in the blood from the coronary sinus.

3. These experiments, therefore, indicate that the Thebesian vessels do form a pathway in the functioning heart by which blood can be conveyed from the heart chambers to the intramural coronary vessels.

4. Under normal conditions the amount of blood conveyed by this pathway appears to be comparatively small, but in some pathological conditions where the coronary arteries are occluded enough blood may find its way into the coronary capillaries to appreciably aid in the nourishment of the heart.

We wish to acknowledge the technical assistance received from other members of the department in carrying out these experiments. We are indebted to Dr. D. J. Cohn and the Department of Chemistry for making the chemical determinations; to Dr. O. Saphir and the Department of Pathology for making the histological sections; and to Dr. K. Howell and the Department of Bacteriology for preparing the bacterial cultures. Dr. O. Saphir and Dr. K. Howell verified the identity of the blood vessels and bacteria. We are grateful to Dr. W. Thalheimer for suggesting the use of bacteria.

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A NOTE ON THE RATE OF CIRCULATION OF CEREBROSPINAL FLUID

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In previous papers, measurements have been given of the rate of flow of cerebrospinal fluid from the aqueduct of Sylvius (4) and of the volumes of the ventricles and subarachnoid space in adult cats (3). These data permit an estimate of the rate at which fluid newly formed at the chorioid plexuses (and ventricular ependyma) displaces older fluid in the ventricles and the subarachnoid space. It is not possible at the moment to give accurately the rate of circulation of the fluid in different parts of the cerebrospinal fluid pathway. In the absence of a direct method for measuring this quantity, recourse must be had to the indirect approach of comparing the rate at which fluid flows from the ventricles with the volumes of the ventricles and the volume of the subarachnoid space.

An average of 12 cc. per day of cerebrospinal fluid has been found to flow from the aqueduct of Sylvius of the etherized cat. Measurement of the combined volumes of lateral and third ventricles gave a value of 0.7 cc. There is, consequently, sufficient fluid formed within these ventricles to renew their total volume 17 times in 24 hours; or new fluid replaces old about once each 85 minutes. Though the difference can only be estimated, the rate of fluid-change in the third ventricle will obviously be greater than in the lateral ventricles. A close approximation to the volume of the third ventricle, based on the combined volumes of the third and lateral ventricles, is probably 0.2 cc. Twelve cubic centimeters of fluid pass through this ventricle in 24 hours; its fluid will therefore be renewed 60 times in 24 hours or once every 24 minutes. The volume of each lateral ventricle may be taken to be approximately 0.3 cc. and the amount of fluid formed within it in 24 hours, 5 cc. Fluid in the lateral ventricles consequently appears to be renewed 17 times in 24 hours or once every 85 minutes.

The fourth ventricle of the cat has a volume of only 0.2 cc. An average of 12 cc. of cerebrospinal fluid in 24 hours reaches it from the aqueduct and to this, from its own chorioid plexus, is added fluid estimated on the basis of the weight of the plexus (4) to be about 3 cc. The fluid in the fourth ventricle consequently is replaced 75 times each 24 hours or at intervals of 20 minutes.

An effort has been made to gain a measure of the rate of circulation in the subarachnoid space. The average volume of the subarachnoid space in the cat has been found to be 3.5 cc.; into this space, from the ventricles, 15 cc. of fluid are delivered every 24 hours. The fluid in the subarachnoid space is consequently renewed every five or six hours. This conclusion agrees remarkably well with the results of Dandy and Blackfan (2) obtained by measuring the rate of absorption of phenolsulphonphthalein from the subarachnoid space. Not too much weight is to be given this substantiation, however, for as pointed out by Becht (1) the phenolsulphonphthalein-method may well be unreliable.

That the rate of circulation of cerebrospinal fluid in the subarachnoid space is of a very low magnitude can perhaps be made more evident in the following way. A segment of subarachnoid space 1 cm. long and with a circumference equal to the average circumference of the space has a volume of about 0.1 cc. Approximately 0.6 cc. of fluid is discharged each hour into the cisterna magna. If the rate of circulation of fluid be equal from the cisterna over the hemispheres and from the cisterna into the spinal subarachnoid space, it follows that fluid will flow upwards and downwards from the cisterna at a rate of 3 cm. per hour or 0.5 mm. per minute. This is an average rate for all portions of the subarachnoid space; wide deviations will occur because of large variations in the volume of different parts of the space. Due to its greater absorbing surface more fluid probably passes through the cranial than through the spinal subarachnoid space. The rate of circulation of fluid over the hemispheres, therefore, is likely in excess, by an undetermined amount, of 3 cm. per hour; and the rate of circulation downwards into the subarachnoid space is likely less than this. These calculations on the rate of circulation of fluid in the subarachnoid space are based on the assumption that a relatively insignificant quantity of fluid arises from the perivascular spaces.

It is to be noted that the findings given here are all based on average values. As has been discussed elsewhere (3), the methods available for determination of the volumes of ventricles and subarachnoid space are not altogether satisfactory and consequently the foregoing statements on rates of fluid change are to be regarded only as approximations.

SUMMARY

Using data on the rate of flow of cerebrospinal fluid from the aqueduct of Sylvius, on the volumes of the ventricles and on the volume of the subarachnoid space in adult cats, calculations have been made of the rate at which new fluid replaces old in the different parts of the cerebrospinal fluid pathway. In the course of 24 hours it appears that fluid in the lateral ventricle is renewed 17 times; in the third ventricle, 60 times; in the fourth ventricle, 75 times; and in the subarachnoid space, between 4 and 5 times.

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COMPARISON OF CHANGES IN CARDIAC AND RESPIRATORY RHYTHMS EFFECTED IN THE DOG BY CHANGES IN PHYSIOLOGICAL CONDITIONS^{1,2}

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The experiments described in this paper were designed to study the early effects of low alveolar oxygen, of high alveolar carbon dioxide, of mechanical asphyxia, and of intravenous injections of sodium cyanide, sodium sulphide, sodium bicarbonate and sodium carbonate on the respiratory and cardiac rhythms of the anesthetized dog. Tentatively, it was assumed that a similarity of response in these physiological rhythms might be indicative of a common mechanism operating at the pace-setter of respiratory movements, and at the pace-setter of cardiac contractions in the same animal (Gesell and Nyboer, 1929b)

METHOD. Nearly a hundred dogs weighing from 7 to 32 kgm. were used. They were anesthetized by injection of about 7 mgm. of morphine sulphate and about 1.0 gram of urethane per kilogram body weight. The trachea was connected to an improved triple unit rebreathing tank (Gesell, 1930) from which the expired carbon dioxide was absorbed by soda-lime (except in carbon dioxide rebreathing experiments). Three to ten per cent oxygen mixtures and five to thirty-eight per cent carbon dioxide mixtures with 22 per cent or more of oxygen were rebreathed at ordinary atmospheric pressures for periods of from one to four minutes. The following solutions were injected in variable amounts into the femoral vein: M/100 sodium cyanide and M/8 sodium sulphide dissolved in physiological saline solution, M/1 sodium bicarbonate (prepared just prior to injection) and M/2 sodium carbonate dissolved in distilled water. Mechanical asphyxiation was produced at the end of inspiration and at the end of expiration by obstructing the tracheal cannula. The blood pressure was recorded with a mercury manometer from the femoral artery.

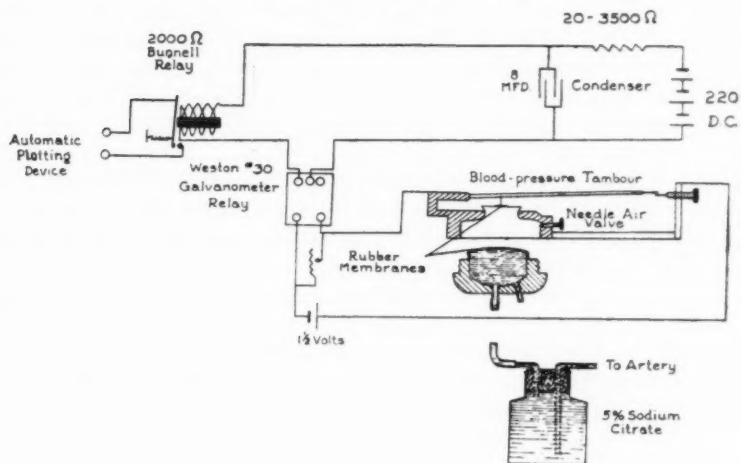
In the earlier experiments the cardiac and respiratory rhythms were counted, the former from the blood pressure tracing, and plotted as ordi-

¹ Preliminary reports. Proc. Soc. Exper. Biol. and Med., 1929, xxvi, 832. Proc., This Journal, 1932, ci, 80.

² Submitted in partial fulfillment of the requirements for the degree of Doctor of Science.

nates against time as abscissae on the kymograph tracings, but in more recent experiments the two sets of rhythmic changes were plotted automatically and simultaneously by a special device of Gesell (1930).³ The closing of an electrical key mounted on a mean blood pressure tambour

PULSE BEAT CONTACT DEVICE AND CIRCUIT



RESPIRATORY MOVEMENT CONTACT DEVICE AND CIRCUITS

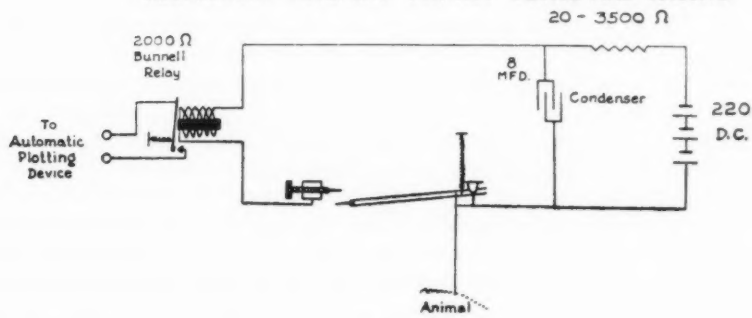


Fig. 1

activated the cardiac rhythm plotter through a system of relays during each diastole of the heart, and the closing of an electrical pneumograph key activated the respiratory rhythm plotter during each inspiratory

³ Demonstrated at the meetings of the American Physiological Society March, 1930.

movement. The diagrams of the electrical circuits involved are self explanatory (see fig. 1). Electrolytic condensers were employed to provide momentary activation of the plotting devices and to avoid the objectionable fusion of contacts. A further advantage of this method was obtained in that positive contacts were assured, as the electrical keys could be closed during any fraction of diastolic or inspiratory time without impairing the operation of the plotting devices. On the fixed records, points representing heart rhythm were connected by a continuous line and points representing respiratory rhythm by a broken line. An upward slope of either curve indicates an increase in rhythm and a downward slope a decrease in rhythm. A comparison of both rhythms was thus obtained.

Both the nervous and humoral control of the heart and respiration were altered. The various combinations of these factors are designated thus:⁴

1. S.X.A.: The intact animal; i.e., the stellate ganglia, the vagus nerves and adrenal glands intact.

2. (S)X.A.: Bilateral stellate-excision, the vagus nerves and adrenals intact.

3. S(X)A.: Bilateral vagotomy (either excision or adequate cold block of the vagus nerves; sometimes one vagus cut, the other cold blocked), the stellates and adrenals intact.

4. (S)(X)A.: Denervated preparation with adrenals intact; i.e., combined bilateral vagotomy and stellate-excision, the adrenals intact.

5. S.X.(A).: Bilateral adrenalectomy,⁵ the vagus and stellate innervations intact.

6. (S)X(A).: Bilateral stellotomy and adrenalectomy, the vagus innervation intact (with one or both vagus nerves functional).

7. S(X)(A).: Bilateral vagotomy and adrenalectomy, the stellate ganglia intact.

8. (S)(X)(A).: Denervated adrenalectomized-preparation; i.e., combined bilateral stellate-excision, vagotomy and adrenalectomy.

For these variations of physiological condition the vagus nerves were either cold blocked or sectioned in the cervical region. The stellate ganglia were excised by a cervical or axillary approach. The adrenals were dissected retroperitoneally and large curved forceps were then clamped over

⁴ In the paper and tables parentheses are used and in the illustrations circles are circumscribed around the letters S, X and A.

⁵ In contrast to Vincent and Thompson's observations (1930) on cats, adrenalectomy in the dog with innervations intact usually resulted in tachycardia and a rise in blood pressure. Subsequently there was a gradual lowering of the blood pressure. Abnormally low blood pressure and a lower heart rate developed following additional excision of the stellate ganglia. A marked fall in blood pressure was likewise noted if adrenalectomy followed stellate ganglia removal. Similar lowerings of blood pressure and heart rate were obtained with and without vagotomy.

each adrenal gland to occlude the venous and arterial supply and to crush the innervation. The clamping of the structures of the hilum was confirmed by autopsy observations.

In an (S)(X)(A) preparation the heart is freed of its adrenal gland control and of its major extrinsic accelerating and inhibiting nervous control. In this connection attention should be called to the experiments of Cannon and his co-workers (1926), who have shown that stellotomy does not remove all sympathetic connections to the heart in recovery experiments on the cat. With "complete" cardiac denervation and removal of all known cardio-accelerator agencies a slow increase in cardiac rate, which was attributed to "sympathin," followed psychic stimulation (Newton, Zwemer and Cannon, 1931). In unanesthetized dogs with denervated adrenals and "complete" cardiac denervation McIntyre (1931) did not find a change in heart rate in response to slight animal movements. Other experimental evidence of Cannon and his associates (1921, 1924, 1927), indicates that full anesthesia (with ether, urethane, or chloralose) greatly reduces (to within 1 or 2 beats per minute) and often abolishes the phenomenon of an increased heart rate on stimulation of the muscles and the sympathetic system in cat preparations in which adrenal denervation, vagotomy and stellate-excision were performed. Thus experiments indicate that changes in heart-rate in the (S)(X)(A) preparation are probably the result of direct chemical action.

Denervation of the pace-setter of respiratory movements is incomplete in the present experiments. As is well known, vagotomy removes important influences of respiratory reflex-control. The effects of stellate ganglia excision are less obvious.

Among the important factors, which may influence respiration in the so-called denervated-preparation, is the carotid-sinus reflex. The respiratory response to various chemical changes in the blood-stream is very much affected by carotid-sinus denervation (Bouckaert, Dautrebande, and Heymans, 1931; and Gesell and Owen, 1931; Winder, Owen and Gesell, 1932; Bernthal, 1932). This reflex offers an additive effect to that of the central stimulation of respiration brought about during chemical change in the animal.

RESULTS. The cardiac and respiratory rhythmic changes developing soon after the introduction of experimental procedures fall roughly into four general types diagrammatically represented in figure 2. Type I shows a primary acceleration of both cardiac and respiratory rhythms and slowing with recovery; type II shows a primary slowing of both rhythms; type III curve shows a slowing of heart rhythm and acceleration of respiratory rhythm; type IV curve shows an acceleration of cardiac rhythm and a decreased respiratory rhythm. Sometimes a change in rhythm failed to appear. Sometimes, during the return toward the normal rate, one or

both rhythms continued to decrease or increase to a rate below or above the basal level, before returning permanently to the basal rate.

The general data obtained with respect to cardiac and respiratory rhythms under each experimental condition are summarized in table 1. Vagotomy and stellate-excision are indicated by the column headings (S).(X)., S.(X)., (S).X., S.X., while the condition of the adrenals is indicated by the A or (A) in the extreme left column headed experimental procedure. The type of reaction, the percentage and number of similar reacting dogs are listed. Table 2 supplements table 1 and gives the average cardiac and respiratory rhythms. Part I of this table lists the average basal rates of each rhythm before experimental procedures in each type of preparation. Part II gives the average percentage change in

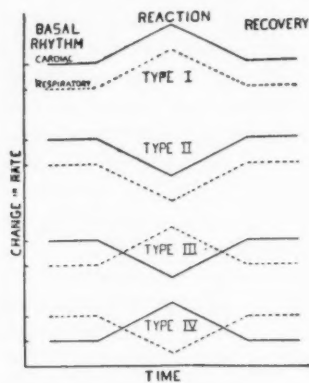


Fig. 2. Diagram representing the four types of changes in cardiac and respiratory rhythms obtained in these studies. Upstroke of either curve indicates acceleration and downstroke indicates slowing of the rhythm. Throughout the paper, similar changes are referred to as types I, II, III, and IV.

primary cardiac and respiratory rhythm, i.e., response above or below the basal rates. Only predominant reactions are averaged in these data. Comparison of these averages is a qualitative one as the injection of solutions and the administration of gases were not standardized. Typical semi-schematic drawings of predominant cardiac and respiratory reactions to given experiments are presented in figures 3, 4, and 5.

In general when the (S)(X)(A) preparations were subjected to experiment, a constant type of response was obtained. In contrast to this, the intact animal with complete innervation and functional adrenals (S.X.A) showed a decidedly variable response. (See table 1.)

Specific results are presented in the order shown in table 1.

A. *The effect of low oxygen on the cardiac and respiratory rate.* (See fig. 3, and tables 1 and 2.) Various workers have reported the effects of

low alveolar oxygen on the cardiac and respiratory rhythms of mammals. In a preliminary paper, Gesell and Nyboer (1929a) have reported comparable accelerations in both rhythms during low alveolar oxygen with the vagi intact or cut, the stellates intact or severed, or both stellates and vagi excised, the adrenals being intact in all preparations. These observations confirmed the findings under some of the above conditions by Paul Bert (1878), Gregg, Lutz and Schneider (1920), Jarisch and Wastl (1926), Mathison (1910), and Somervell (1925). Slowing of the heart during

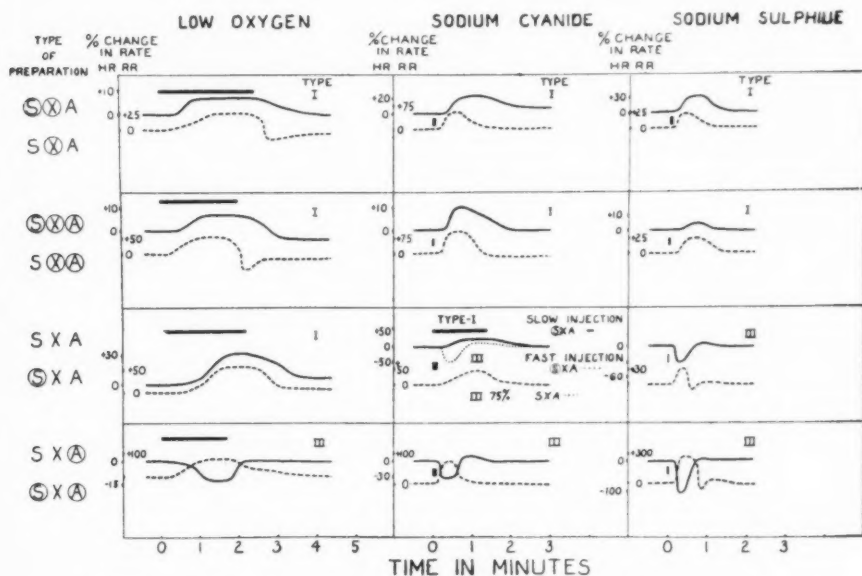


Fig 3. Graphs of predominant changes in cardiac (—) and respiratory (---) rhythms effected by low alveolar oxygen, and the intravenous injections of sodium cyanide and sodium sulphide. The experimental conditions are indicated in the left margin; the duration of gaseous administration by a horizontal line above each pair of curves, and duration of injection by a short vertical line between the curves. Percentage change (\pm) in each rhythm is plotted on the ordinates against time in minutes on the abscissae.

anoxemia, however, was observed by Takeuchi (1925), and Gremels and Starling (1926); the former in cats with cardiac innervation intact, artificial ventilation and urethane-ether narcosis, the latter in denervated dog heart-lung preparations.

In the present experiments with the adrenals intact low alveolar oxygen usually accelerated both the heart and respiratory rhythms as in curve type I with all types of innervation, S.X.A., (S).X.A., S.(X).A., (S)(X).A. (See fig. 3.) In one out of 24 S.X.A. dogs there was a late but distinct

TABLE I
Tabular summary of early respiratory and cardiac rhythmic responses to experimental procedures

EXPERIMENTAL PROCEDURE	(s)(x)			(s)(x)			(s)(x)		
	Type of reaction	Similarly reacting dogs		Type of reaction	Similarly reacting dogs		Type of reaction	Similarly reacting dogs	
		Num-ber	Per-cent		Num-ber	Per-cent		Num-ber	Per-cent
Low O ₂ ,	I	6	100	I	9	100	I	6	100
	I	10	100	I	10	100	III I No H.R. Change	8 2 1	80 20
	I	17	100	I	16	94	I (slow) III (fast)	3 9	100 100
	I	12	100	I	9	100	III	8	100
NaCN,	I	23	96	I	10	100	III	6	84
	II	1	4	No H.R. Change	2		II	1	16
	I	4	100	I	4	100	III	5	100
	III II	11 4	73 27	II III	6 3	67 33	III	7 4	54 31
CO ₂	I	10	100	III	10	100	I	2	15
	I	10	100	III	10	100	IV	1	4
	I	10	100	III	10	100	III	14	100
	I	10	100	III	10	100	III	14	100

(A)...	III	10	100	III	10	100	III	6	100	III	14	100
NaHCO ₃	A....	III	16	88	III	7	78	III	5	63	III	42
	I	II	2	8	II	2	22	II	3	37	I	33
	I	I	1	4							II	25
	(A)...	III	5	72	III	4	100	I	2	50	III	100
Na ₂ CO ₃	I	I	2	28				II	1	25		
	(A)...	III	18	86	IV	5	72	IV	6	84	IV	86
	I	I	3	14	I	2	28	II	1	16	I	14
	(A)...	IV	7	86	IV	4	100	IV	5	72	II	50
Mechanical asphyxia	I	I	1	14				I	2	25	IV	33
	(A)...	III	4	44	III	5	100	III	1	13	III	17
	I	I	3	33				II	6	100	II	53
	(A)...	> R.R.	2	23	> R.R.	2	25	II	4	67	IV	27
end of ex- piration	I	> R.R.	3	43	III	2	50	III	2	33	III	10
	(A)...	0 H.R.	3	43	< R.R.	1	25	II	2		I	2
	I	< R.R.	1	14	0 H.R.	25						10
	(A)...	> R.R.	8	57	III	5	62	III	5	100	II	60
End of in- spiration	I	I	4	29	I	2	25	II			IV	31
	(A)...	III			> R.R.	1	13				> R.R.	6
	I	I			0 H.R.						0 H.R.	
	(A)...	> R.R.	7	100	III	3	75	III	3	50	II	100
	(A)...	0 H.R.			> R.R.	1	25	II	2	33		
		0 H.R.			0 H.R.			> R.R.	1	17		

The following additional notations have been used: >, acceleration; <, slowing; and 0, no change in rhythm, R R., respiratory rate; H.R., heart rate.

* Following an initial acceleration in heart-rate, a slowing below the basal cardiac rate resulted before the end of the low oxygen administration.

TABLE 2

EXPERIMENTAL PROCEDURE	ADRENALS INTACT										ADRENALS CLAMPED									
	(S) (X) A		S (X) A		(S) X. A.		S. X. A.		(S) (X) (A)		S (X) (A)		(S) X (A)		S. X (A)		H. R.		H. R.	
	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.	H. R.	R. R.
Part I—Average basal rate per minute before experiment																				
	149	13	211	10	138	20	117	18	137	12	225	12	133	17	212	19				
Part II—Average per cent change of rate																				
Low O ₂	+8.7	+26	+7	+75	+19	+42	+30	+62	+7.0	+65	+7	+42	-18 +6	+100	-17 +7	+145				
NaCN.....	+18.6	+46	+8	+90	-42 +22	+37	+56	+145	+10.0	+59	+5	+55	-33	+98	-30	+148				
Na ₂ S.....	+16.0	+59	+7	+75	-51	+66	-42	+145	+3.0	+84	+8	+62	-29	+32	-46	+118				
CO ₂	-10.0	+27	-10	+20	-17	-15	-24	+17 -43	-12.0	+40	-10	+23	-14	+40	-17	+32				
NaHCO ₃	-7.7	+14	-4	+30	-12	+17	-20	+35	-6.3	+12	-3	+14	-8	+20	-11	+15 -18				
Na ₂ CO ₃	+19.0	-40	+13	-31	+22	-38	+48	-44	+20.0	-44	+6	-24	+10	-51	+51 -18	-42				
Mechanical asphyxia end of expiration	+9.0 -6.0	+45	-3	+56	-26	-34	-20 +19	-26 0	-3.0 0	+25	-3 0	+50 0	-9	+35 -50	-28	±5				
End of inspiration...	+13.0 -3.0	+40	-3 +5	+50	-30	-45	-26 +18	-50	0	+35	-5 0	+30 0	-8	+50 -30	-18	-46				

slowing of heart rhythm following an initial acceleration during lowered alveolar oxygen. (See footnote* table 1.)

With adrenals clamped in (S)(X)(A) and S(X)(A) preparations primary accelerations of heart and respiratory rhythms were obtained as in all dogs with adrenals intact. (See fig. 3.) The results are similar to the effects of bulbar anemia in which temporary occlusion of the head arteries produced cardiac acceleration in vagotomized, and in combined vagotomized and stellate-excised preparations in normal or adrenalectomized cats (Coombs, 1924, 1925, 1926).

The reaction to low alveolar oxygen of dogs with adrenals clamped, but with the vagus nerves intact S.X.(A), was frequently different from the observations described above. In these instances the usual acceleration of heart rate was frequently replaced by a slowing of rate during the administration period. An initial increase in respiratory rate generally occurred.⁶ Specifically, 80 per cent of ten (S)X(A) preparations and 61 per cent of eighteen S.X.(A) preparations reacted according to type III (see fig. 3, tables 1 and 2). The remaining reactions were of type I. In most exceptions additional anesthesia was injected before the experiment was begun in order to eliminate irregularities due to extraneous stimuli.

Thus, comparison of experiments in adrenalectomized-preparations, either S.X.(A) or S(X)(A), with those in adrenal-preparations, either S.X.A. or S.(X).A, indicates that the adrenal glands play an important accessory rôle in control of cardiac rhythm during anoxemia. The integrity of the vagus innervation was necessary to the cardiac inhibition frequently obtained in adrenalectomized-preparations.

With these observations in mind the results of sodium cyanide and sodium sulphide on cardiac and respiratory rhythms may be considered.

B. *The effects of intravenous injections of sodium cyanide on heart and respiratory rhythms* (see fig. 3, tables 1 and 2). Little attempt has been made to correlate the changes in cardiac and respiratory rhythms incurred by injection of sodium cyanide in animal or man. Geppert (1889) demonstrated that hydrocyanic acid action is essentially one of asphyxiation, since it acts by decreasing the oxygen absorbed by the tissues and also the carbon dioxide produced by them. Bodine (1924) claims that HCN in acid, neutral, or slightly alkaline media produces intra-cellular acidity in plants and in artificial cells made of living frog skin because of the rapid penetration of HCN molecules. The correlation of continuous changes in numerous factors of respiratory control following intravenous injection of

* One adrenalectomized dog invariably gave a decrease in respiratory rhythm regardless of the experimental procedure, i.e., low O_2 , Na_2S , $NaCN$, CO_2 , $NaHCO_3$, Na_2CO_3 in S.X., (S.)X., S.(X.), (S) (X) preparations produced a slowing of respiratory rate. (Data are not included in table 1.) Severe operative procedures and low resistance of the animal might be the cause of this exception.

sodium cyanide by Gesell, Krueger, Gorham, and Bernthal (1930) is of considerable importance in an interpretation of cyanide action. These workers find that lowered alveolar oxygen and NaCN produce similar metabolic changes in the dog. In a preliminary report, Gesell and Nyboer (1929a) indicated that cyanide augments both cardiac and respiratory rhythms in dogs with adrenals intact in very much the same manner as low alveolar oxygen.⁷

A tabular summary of our experiments on the effects of intravenous cyanide injections in normal adrenal and adrenalectomized dogs is also shown in tables 1 and 2. When the adrenals were intact, sodium cyanide usually produced acceleration of heart and respiratory rates in (S)(X)A, S(X)A, (S)X.A., and S.X.A. preparations with few exceptions. With complete cardiac innervation, S.X.A., 75 per cent of the dogs gave type I response; the remaining 25 per cent were of type II, III, or IV. In (S) X.A. preparations type III response was obtained with rapidly injected cyanide; but slow injections in the same preparations brought about type I response as in the intact animal. (See fig. 3, tables 1 and 2.)

When the adrenals were clamped, the vagus nerves cut, and the stellate ganglia either intact or removed, S.(X)(A) or (S)(X)(A), the responses to sodium cyanide were invariably of type I, i.e., an increase in the cardiac and respiratory rates.

If, however, the vagus nerves were intact, S.X.(A) or (S)X(A), the adrenalectomized dog responded differently to sodium cyanide. These responses resembled those of low oxygen under similar conditions, i.e., a decreased heart rate and an increased respiratory rate resulted as in type III. (See fig. 3 and table 1.) Only one exception, a type I response, was recorded from observations in nineteen dogs. This one dog, however, on rapid injection of sodium sulphide gave a response typical of cyanide, which as will be seen below resembles the typical sulphide response under these conditions.

C. *The effects of sodium sulphide injections.* (See fig. 3, tables 1 and 2.) A detailed report (Gesell and Nyboer, 1929b) of the effects of sodium sulphide in dogs with intact adrenals, showed that intravenous injections in denervated preparations generally produced an increase in cardiac and respiratory rhythms. In experiments with intact innervation, differences in response were evident. In the present report these data are included and correlated with further experiments.

Sodium sulphide effects may be grouped into type I or type III depending on the integrity of the vagus innervation. Type I reaction almost

⁷ Loevenhart, Schlomovitz and Seybold (1922) observed that twenty-five cyanide injections usually less than 0.75 cc. of 0.02 NaCN per kilo in normal, *unanaesthetized* dogs (vagus nerves and stellate ganglia intact) resulted in early cardiac slowing twenty times, cardiac acceleration two times, and no cardiac effect three times.

invariably occurred in vagotomized dogs, whether the stellate ganglia were intact or excised, i.e., (S)(X) or S(X). Type III always occurred in dogs with vagus innervation, whether the stellate ganglia were intact or excised, i.e., (S)X, or S.X. preparations. These results were usually the same before and after adrenalectomy. (See fig. 3, tables 1 and 2.)

Comparing rapid injections of cyanide and sulphide in S.X.A. preparations, it is seen that, whereas cyanide usually accelerated the heart, sulphide usually slowed it. Respiratory rhythm was accelerated by both substances. Under all other conditions, both rhythmic responses showed great similarities with sulphide and cyanide injections. (See fig. 3.)

In view of the similarity of the effects of sodium sulphide, sodium cyanide, and low alveolar oxygen, especially in adrenalectomized dogs, it is sug-

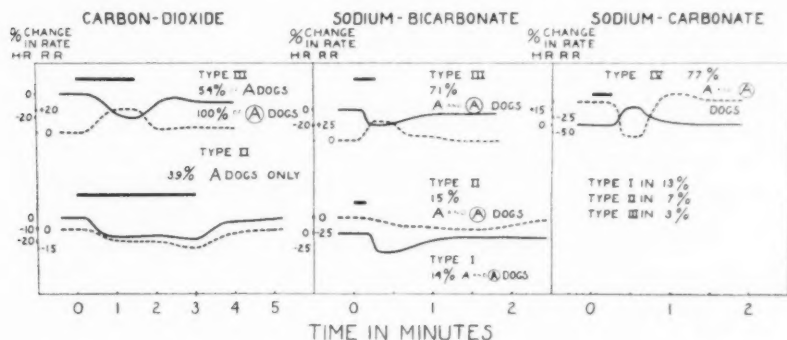


Fig. 4. Graphs of predominant changes in cardiac and respiratory rhythms effected by high alveolar carbon dioxide, and intravenous injections of sodium bicarbonate and sodium carbonate. The preparations with adrenals intact are represented by A, and adrenalectomized preparations by (A).

gested that the chemical changes responsible for the changes in cardiac and respiratory rhythm control may be the same. The irritability of the bulbar vagus centers to these substances, and the inhibitory influence of these substances on the heart rate was most clearly demonstrated in adrenalectomized animals.

D. *The effects of rebreathing carbon dioxide in gas mixtures containing 22 per cent or more of oxygen.* (See fig. 4, tables 1 and 2.) Although considerable work has been done with carbon dioxide as a respiratory stimulant, it was found profitable to repeat many observations on the anesthetized dogs under various conditions of nervous and adrenal gland control. In man, Schneider and Truesdell (1922) found that respiratory rate gradually increased with high alveolar CO_2 . Haldane (1922) states that high alveolar carbon dioxide chiefly increases the depth of breathing and

causes only a moderate increase in frequency. From several hundred experiments on the dog, Gesell (1929a) observed that the common result of high alveolar carbon dioxide and low alveolar oxygen was to produce an increase in rate and depth of breathing. On the whole lowered alveolar oxygen accelerated the rate of respiratory movements more than did increased alveolar carbon dioxide pressure. These experiments indicated that the difference in respiratory rhythmic response to low oxygen and excess carbon dioxide is one of degree.

Waller and Sowton (1896) demonstrated the slowing effects of carbon dioxide on the excised heart of turtle and frog; Jerusalem and Starling (1910) in heart-lung preparations of the dog. Yandell Henderson (1908), working with dogs in which cardiac innervation was intact, found that an inverse relation existed between heart rate and carbon dioxide content of the arterial blood. According to Schneider and Truesdell (1922) the heart rate in man was usually accelerated or unaffected by high alveolar carbon dioxide.

In the present series of experiments with adrenals intact, high alveolar carbon dioxide caused an acceleration or a slowing of respiratory rhythm, whereas the heart was almost invariably slowed (see table 2, part II). A study of the carbon dioxide data in table 1 shows that carbon dioxide elicited all four types of rhythmic reactions; type III occurred in 73 per cent of (S)(X)A preparations, in 33 per cent of S.(X)A. preparations, in 54 per cent of (S)X.A. preparations, and in 59 per cent of S.X.A. preparations. Type II occurred in 27 per cent of (S)(X)A preparations, in 67 per cent of S(X)A preparations, in 31 per cent of (S)X.A. preparations, and in 29 per cent of S. X. A. preparations. Type I reaction occurred only in 15 per cent of the (S)X.A. preparations, and in 8 per cent of the S.X.A. preparations. Type IV occurred only once in the whole series of carbon dioxide observations (in an S.X.A. preparation).

In adrenalectomized dogs, type III reaction invariably occurred if gross tissue abuse was avoided during the operations (see table 1 and fig. 4 and footnote on p. 213). Further experiments, however, must supplement these data to determine the quantitative relations probably existing between the normal and adrenalectomized preparations.

Respiratory acceleration occurred in 61 per cent of all carbon dioxide experiments and slowing occurred in 39 per cent. Cardiac slowing occurred in 93 per cent and acceleration in 7 per cent of all carbon dioxide experiments. The significance of the occurrence of two major types of acute rhythmic reaction (i.e., types III and II), in animals with adrenals intact, and only type III reaction in adrenalectomized dogs, is not understood. (See table 1 and fig. 4.)

E. *The effects of sodium bicarbonate.* (See fig. 4, tables 1 and 2.) The effects of intravenous injections of sodium bicarbonate on pulmonary ventilation, reflex and muscular activity, salivary secretion, and on acidity and

volume flow of lymph and blood have been studied by Gesell and McGinty (1927); Glazer (1929), Winkler (1930), Gay (1931); Eddy (1930); Bronk and Gesell (1927), Gesell (1929b). It has been pointed out that the effects of sodium bicarbonate are diametrically opposite in action to sodium carbonate. That such differences are possibly attributable to opposite effects on the blood carbon dioxide pressure, is supported by experimental data (Gesell, 1923; Gesell and Hertzman, 1926; Gesell and McGinty, 1927).

In the present series of experiments with intravenous injections of NaHCO_3 , acceleration of respiratory rhythm generally resulted, whereas heart rate was generally slowed. (See fig. 4, tables 1 and 2.)

With adrenals intact, type III and type II reactions occurred most frequently with sodium bicarbonate injections and type I least (see table 1 and fig. 4).

In adrenalectomized animals, type III was predominant in (S)(X)(A), S(X)(A), and S.X(A) preparations with bicarbonate injection. Conclusions can not be drawn from the results on (S)X(A) preparations as there are only four experiments and the results of these are variable. (See tables 1 and 2.)

In general, the changes obtained in both rhythms with sodium bicarbonate suggest a great similarity to those obtained with high alveolar carbon dioxide. Summarizing the seventy bicarbonate experiments, type III occurred in 71 per cent, type II, in 15 per cent, and type I in 14 per cent. Respiratory rhythm was accelerated in 85 per cent of these experiments, whereas cardiac rhythm was slowed in 86 per cent.

F. *The effects of sodium carbonate.* (See fig. 4, tables 1 and 2.) That intravenous injection of sodium carbonate generally causes a decrease in pulmonary ventilation, a lowering of the blood pressure, and a decrease in acidity of the blood and tissues, is accepted. The diametrically opposite physiological effects on respiration of sodium carbonate to those of sodium bicarbonate, are possibly due to the opposite effects on the blood carbon dioxide pressure caused by these experimental agents (Gesell, 1923; Gesell and Hertzman, 1926a; Gesell and McGinty, 1927).

In the present experiments, heart rhythm usually increased while respiratory rhythm usually decreased under the eight conditions of cardiac innervation and adrenal condition. Adrenalectomy had little effect upon the results, as reaction type IV occurred in 82 per cent of all adrenal dogs and in 70 per cent of all adrenalectomized dogs. (See tables 1 and 2 and fig. 4.)

Summarizing the sixty-nine Na_2CO_3 observations under the different conditions outlined, and in different dogs, we find that reaction type IV occurred in approximately 77 per cent of the experiments; type I in 13 per cent; type II in 7 per cent, type III in 3 per cent.

G. *The effects of mechanical asphyxia produced at the end of expiration and*

at the end of inspiration. (See fig. 5, tables 1 and 2.) The effects of mechanical asphyxia on the hydrogen ion concentration of the cerebro-

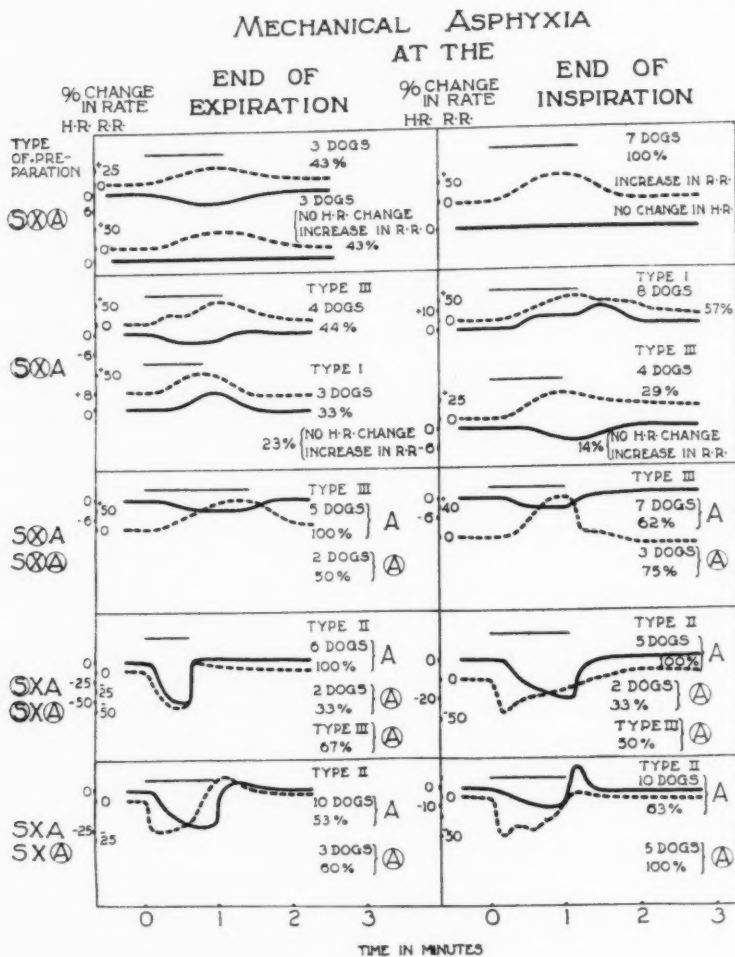


Fig. 5. Graphs of cardiac and respiratory rhythm changes effected by mechanical asphyxia at the end of expiration and at the end of inspiration in the various types of preparations indicated on the left margin of the figure.

spinal fluid, arterial and venous blood, on the reflex response of the tibialis anterior muscle, and on segmental respiratory movements of the dog, have been studied (Gesell and Hertzman, 1927, 1928; Glazer, 1929; Gesell and

Moyer, 1933). The present report considers only the effects on cardiac and respiratory movement rates following asphyxiation for periods of 30 seconds or longer following a normal inspiratory or expiratory movement.

In denervated preparations, (S)(X)A, mechanical asphyxia at the end of expiration produced reactions of type III, 44 per cent; type I, 33 per cent; and in 23 per cent an increase in respiratory movement rate but no change in heart rate when the adrenals were intact. In adrenalectomized and denervated preparations, (S)(X)(A) type III occurred 43 per cent, but in the remaining 67 per cent no heart rate change was demonstrable while respiratory movements were either accelerated or retarded during the asphyxial period. With asphyxiation at the end of the inspiration, however, type I occurred most commonly (57 per cent of fourteen dogs with adrenals intact (S)(X)A; type III occurred 29 per cent, and in 14 per cent the heart rate did not alter while respiratory movements accelerated. In adrenalectomized preparations, (S)(X)(A), heart rate was never observed to retard or accelerate while respiratory efforts were invariably accelerated during asphyxia at the end of inspiration.

In vagotomized S(X)A or S(X)(A) preparations reaction type III was predominant. Occasionally heart rate did not change during the asphyxial period. Retardation of respiratory rate sometimes occurred, but acceleration of heart rate, rarely.

When the stellate ganglia were excised either type of asphyxia invariably effected reaction of type II when the adrenals were intact, (S)X.A. In adrenalectomized (S)X(A) preparations type III was predominant but type II was not uncommon. (See table 1.) The irritability of the vagi may have been partially altered here due to previous experimenting with cold block. This may explain the occasional accelerating respiratory rhythm in this procedure.

In normally innervated dogs, S.X(A) and S.X.A., reaction type II was predominant during either type of asphyxia if the adrenals were functional or clamped. (See table 1.) Types 1, 3 and 4 were also observed. In S.X.A. experiments acceleration of the heart was obtained when the basal heart rate was low (approximately 80) and slowing of the heart when the basal rate was higher (approximately 127).

Summarizing, it appears that the integrity of the vagus was necessary to produce a primary reflex respiratory slowing as observed in S.X.A., S.X.(A), (S)X.A. and (S)X(A) experiments. Respiratory acceleration usually occurred when the vagi were cut or excised (S(X)A, S(X)(A), (S)(X)A and (S)(X)(A)). Slight slowing of the heart takes place in spite of predominant sympathetic innervation in S(X)A and S(X)(A) experiments. Marked cardiac slowing usually occurred in S.X.A., (S)X.A., S.X.(A), and (S)X(A) experiments. Cardiac acceleration sometimes observed in (S)(X)A experiments with either type of asphyxia was not elicited

in adrenalectomized preparations (S)(X)(A). Here, mechanical asphyxia at the end of inspiration never altered heart rate; whereas mechanical asphyxia at the end of expiration sometimes slowed it. Thus it appears that the acceleration in (S)(X)A experiments was of adrenal gland origin. Slowing was probably due to a greater accumulation of metabolic products at the cardiac pace-setter during mechanical asphyxia at the end of expiration as compared with asphyxia experiments at the end of inspiration. There appears to be a close analogy with similar effects under those conditions produced by carbon dioxide administration.

CORRELATION AND DISCUSSION. The corresponding experimental results are briefly correlated according to type of reaction and type of preparation in table 3. Only the reactions which occurred most frequently in the various preparations are considered in this table. In reactions I and II the change of rate in cardiac and respiratory movements are parallel (I shows acceleration and II slowing of both rhythms). In reaction III and IV there is an opposite response of cardiac and respiratory rhythm (IV shows early acceleration of heart and early slowing of respiratory movements, whereas III shows early slowing of the heart and early acceleration of respiratory movements).

The results in the denervated preparations, (S)(X)A and (S)(X)(A), are probably the best criteria for observations of the most elementary effects produced by the experimental agents studied. Therefore, in view of the similarity of reaction of both rhythms to low O_2 , Na_2S , and $NaCN$, it is suggested that there may be common fundamental factors responsible for acceleration of the heart and respiration in these preparations. On the other hand, in view of the dissimilarity between heart and respiratory rhythms when CO_2 and $NaHCO_3$ and Na_2CO_3 are the experimental agents, it appears that the fundamental factors controlling the rhythms are different or that additional factors are involved. The apparent antagonistic effects on the heart rate of these two sets of procedures, (i.e., acceleration by low O_2 , Na_2S , $NaCN$, and Na_2CO_3 , and slowing by CO_2 , $NaHCO_3$), cannot be ascribed to an extrinsic nervous activity, since the heart is denervated. Neither can it be ascribed to the liberation of adrenal gland secretions, since similar results are usually obtained in completely adrenalectomized dogs, nor can it be ascribed to known accessory hormones, which are not activated in fully anesthetized dogs under urethane narcosis (Cannon, 1931). Therefore, the only known agent left which may determine the change in rhythmic activity is a direct chemical or physico-chemical control of the cardiac pace-setter. As regarding respiratory movements, we may have a direct, a reflex chemical, and a reflex mechanical control of the pace-setter. At present, however, there is no immediate evidence as to the exact nature of the controlling mechanism. Its determination is beyond the scope of these experiments. Gesell (1926) has tentatively

TABLE 3
Tabular correlation of predominantly similar experimental results according to reaction type and type of preparation

TYPE OF REACTION	ADRENALS INTACT				ADRENALS CLAMPED			
	(S)(X)A	S(X)A	(S)X.A.	S.X.A.	(S)(X)A	S(X)A	(S)X(A)	S.X(A)
I	Low O ₂ NaCN Na ₂ S +*(E.E.) *(E. I.)	Low O ₂ NaCN Na ₂ S	Low O ₂ NaCN (slow)	Low O ₂ NaCN	Low O ₂ NaCN Na ₂ S	Low O ₂ NaCN Na ₂ S		
II		CO ₂	(E.E.) (E.I.)	E.E.) *(E.I.)			*(E.E.) *(E.I.)	(E.E.) (E.I.)
III	CO ₂ NaHCO ₃ *(E.E.) *(E.I.)	NaHCO ₃ (E.E.) (E.I.)	NaCN (fast) Na ₂ S CO ₂ NaHCO ₃	Na ₂ S CO ₂ NaHCO ₃	CO ₂ NaHCO ₃ (E.E.) (E.I.)	Low O ₂ NaCN Na ₂ S CO ₂ *(NaHCO ₃) *(E.E.) *(E.I.)	Low O ₂ NaCN Na ₂ S CO ₂ NaHCO ₃	
IV	Na ₂ CO ₃	Na ₂ CO ₃	Na ₂ CO ₃	Na ₂ CO ₃	Na ₂ CO ₃	Na ₂ CO ₃	Na ₂ CO ₃	*(Na ₂ CO ₃)

+E.E. indicates mechanical asphyxia at end of expiration; E.I. indicates mechanical asphyxia at end of inspiration.

† Heart rate remained unchanged during asphyxial periods.

* Uncertain. See table 1.

proposed an electrochemical hypothesis of physiological rhythm control, which includes oxidation-reduction potentials as a factor. The similar or dissimilar response of the cardiac pace-setter and the respiratory pace-setter to apparently similar chemical stimuli, have been pointed out with special reference to the denervated heart preparations. The parallelism in cardiac and respiratory rhythms in denervated preparations, following low oxygen, sodium cyanide and sodium sulphide administrations, support the postulate of a common mechanism of rhythm control. The lack of parallelism of both rhythms in (S)(X)(A) preparations following mechanical asphyxia at the end of expiration, carbon dioxide, sodium bicarbonate and sodium carbonate administrations, do not directly support this postulate.

During low alveolar oxygen and on injection of sodium cyanide, the auxiliary activity of the adrenal glands on cardiac and respiratory rhythms was evident in preparations with vagi and adrenals intact, (S)X.A. or S.X.A., particularly if these results were compared with those obtained in adrenalectomized-preparations with vagus innervation intact, (S)X(A) or S.X(A). The accelerated heart, (as in type I reaction), typical of the adrenal-preparation, was seldom observed in the adrenalectomized-preparation during these procedures; hence impaired oxidations resulted in inhibition of the heart through the vagus, due to the absence of the accelerating component of the adrenal gland. This inhibition was especially true in (S)X(A) preparations since both nervous and adrenal accelerating components were lacking. Vagotomy abolished this cardiac slowing phenomenon.

SUMMARY

This study attempts to compare changes in cardiac and respiratory rhythms effected by common changes in physiological conditions in the dog. The effects of low alveolar O_2 , high alveolar CO_2 , mechanical asphyxia, and of injections of NaCN, Na_2S , $NaHCO_3$, and Na_2CO_3 were recorded in eight types of preparations, in which the function of the vagus nerves, stellate ganglia and adrenal glands was controlled. Morphine-urethane anaesthetized dogs were used.

Carbon dioxide or sodium bicarbonate administrations usually slowed cardiac rhythm in most preparations. These substances either accelerated, slowed, or had no effect on respiratory rhythm. On the other hand sodium carbonate injections usually slowed respiratory rhythm and accelerated cardiac rhythm.

Mechanical asphyxia almost invariably accelerated respiratory rhythm in vagotomized, and in combined vagotomized and stellate-excised preparations with adrenal glands intact or occluded from the circulation. With adrenals intact, the cardiac rhythm was either accelerated, slowed or un-

altered during asphyxia of either type. If, however, the adrenals were occluded, moderately short asphyxias at the end of inspiration never altered the heart rhythm; whereas asphyxia at the end of expiration frequently caused a slight slowing. When the vagi nerves were intact marked slowing of both cardiac and respiratory rhythms resulted most frequently during asphyxia of either type.

Both rhythms were accelerated in vagotomized, or combined vagotomized and stellate-excised preparations, by low alveolar O_2 , NaCN, and Na_2S , whether the adrenals were intact or clamped. When the vagus nerves were intact, rhythmic responses to low alveolar O_2 and to NaCN differed in adrenal and adrenalectomized dogs; i.e., adrenal preparations showed acceleration of both rhythms, whereas adrenalectomized preparations usually showed a retardation of cardiac rhythm and simultaneous acceleration of respiratory rhythm. Vagotomy abolished these differences.

It is concluded that in denervated preparations (combined vagotomy and stellate-excision), the effects of the low alveolar O_2 , NaCN, and Na_2S were very similar on both cardiac and respiratory rhythms. Less agreement in both rhythms was obtained with CO_2 and $NaHCO_3$ administrations and during mechanical asphyxia. Sodium carbonate injections usually produced opposite results in cardiac and respiratory rhythms. Until further evidence is forthcoming, conclusions regarding the similarity of a common mechanism of physiological rhythm control must be postponed.

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HEAVY METAL CATALYSIS IN SMOOTH MUSCLE CONTRACTURE

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The starting point of the present investigation was the observation² that the addition of small quantities of sodium cyanide to the Ringer solution used to perfuse the turtle heart causes a reversible inability of vagus stimulation to stop the heart, and that cyanide added to Tyrode solution in very low concentration reversibly inhibits the action of adrenalin, acetylcholine, pilocarpine, arecolin, and other drugs upon strips of guinea-pig intestine, uterus or other smooth muscle preparations suspended in a Trendelenburg apparatus.

In view of the low concentration of cyanide needed to exhibit these striking effects, the possibility suggested itself that a heavy metal catalyst may be involved generally in the mediation of autonomic impulses to visceral organs.

In the intervening years the heavy metal catalytic mechanisms in cells have been thoroughly investigated, particularly by Warburg and his collaborators, and it has been recognized that the heavy metal catalyst poisoned by cyanide is probably of the nature of a hemin compound. This has been established from the photosensitivity of cells poisoned by carbon monoxide. The absorption spectrum of the catalyst indicates beyond doubt that its active group is a hemin compound. In view of these advances in knowledge of the chemical nature of the catalytic mechanism in cells we have resumed these early experiments and propose in this paper and in future work to investigate the possibility that the response of visceral organs to autonomic nerve impulses and perhaps to humoral stimuli in general requires a catalytic activating mechanism at one or more points in the process.

If a heavy metal hemin-like catalyst is involved one would expect that not only cyanide but also hydrogen sulfide and carbon monoxide would similarly inhibit the response of the tissue to autonomic nerve influence

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² These unpublished observations were made in a series of experiments performed by the senior author in the fall of 1925 in the Physiological Laboratory of Washington University.

and to humoral stimulation. The action of these substances has been studied and it has been found that with strips of intestine and uterus these poisons inhibit the action of adrenalin, acetylcholine, pilocarpine, arecolin, etc. (Schmitt and Nicoll, 1933). Furthermore if the carbon monoxide-treated tissue be illuminated the inhibition disappears and the tissue responds to the reagent as though no carbon monoxide were present. We consider this photosensitivity of the carbon monoxide-treated tissue as crucial to the suggestion that we are dealing with a hemin-like catalyst, although no experiments on the efficacy of various wave lengths were performed especially to establish this identity.

EXPERIMENTAL. Methods and material. As stated, the smooth muscle types investigated in this series of experiments were the intestine and uterus of the rabbit. A few experiments also were performed on rat intestine, the results corroborating those obtained with rabbit intestine.

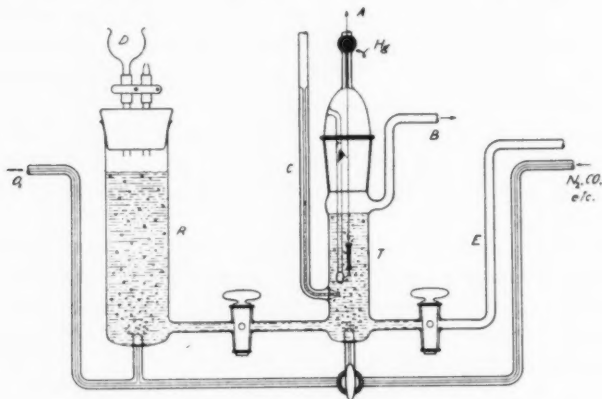


Fig. 1

The tissue was removed immediately after killing the animal and after washing in Tyrode solution was placed in a moist chamber in the ice chest for several hours. The Trendelenburg apparatus for recording movements of excised smooth muscle strips was modified as shown in figure 1, in order to permit the use of gas mixtures of known composition. Sealed to the upper ground joint member is a short capillary tube ending in a small bulb, the thread from the tissue to the recording lever passing out of the tissue chamber through this capillary. The small bulb is filled with mercury, permitting free movement of the string, and acting as a seal to prevent the escape of gas from the chamber. Because of its high surface tension the mercury remains in the form of a globule in the bulb and has little tendency to flow into the capillary or the chamber below. With

this method it is possible to equilibrate the solution in the tissue chamber with gas mixtures of known composition, and at the same time to record satisfactorily the movement of the tissue on the kymographion.

The introduction of drugs or reagents into the tissue chamber *T*, is accomplished by means of capillary *C*. Since the entrance of this capillary into the chamber is below the level of the tissue, the gas bubbles emitted serve to mix the test solution before it reaches the tissue. In this manner the concentrations of the test solution can be controlled within reasonable limits. Exhaust tube, *E*, connected to a vacuum pump permits rapid evacuation of the solution in the tissue chamber. The gas mixture with which the solution is to be saturated is introduced through the lower capillary tube; its flow is controlled by means of the three-way stopcock beneath the tissue chamber. The gas escapes from the tissue chamber through tube *B*, which leads through a water seal to prevent leaks. The whole apparatus is submerged in a water bath and the temperature kept at $37.5 \pm 0.5^\circ\text{C}$.

In the earlier experiments a bicarbonate Tyrode solution was used of the same composition as given by Garry (1928). Preliminary tests showed, however, that the pH of this solution changed during an experiment due to the blowing off of CO_2 . These determinations of pH were made with a glass electrode permitting continuous recording as the experiment progressed.³ The initial pH of the solution ranged from 7.2 to 7.5 but rose to a value of 8.2, as air was bubbled through, at first rapidly and then slowly as equilibrium was approached. Despite its high pH value, however, this bicarbonate Tyrode solution was found to give excellent results, especially in the experiments with sodium cyanide. A few attempts at saturating the air initially with CO_2 as suggested by Thomas (1931) were not successful, probably due to unsatisfactory adjustment of the concentration of magnesium carbonate-phosphate mixture. Since this method would not have been applicable when gases other than air were being used it was abandoned.

To eliminate variation of the pH as a possible factor the experiments were all repeated with phosphate Tyrode solution of the composition given by Garry (1928). The pH of this solution was between 7.1 and 7.2 and did not change during experimentation. In this case it is necessary to exercise care to prevent a rise in pH above 7.2 lest the calcium be precipitated. A further difficulty arises in that HCN is liberated rather rapidly from cyanide solutions at pH values as low as 7.1. The most noticeable difference in the results with the two solutions is that the sustained contracture induced by reagents lasts longer in the bicarbonate than in the phosphate Tyrode solution. Although it is generally believed

³ The form of the glass electrode used was that recently developed by R. K. Skow, the details of which will be published elsewhere.

that phosphate is not as suitable as bicarbonate as a physiological milieu, all the results obtained in the present experiments with the bicarbonate Tyrode solution were also obtainable with phosphate Tyrode solution. Hence, because of the stability of the pH of the phosphate solution all the later experiments were carried out with this modification of Tyrode's solution.

Although the usual parasympathomimetic and sympathomimetic substances were tried, the most satisfactory results were obtained with acetylcholine as a parasympathomimetic stimulant and with adrenaline as a sympathomimetic stimulant. These were used in concentrations of the order of 1×10^{-6} . All reagents were prepared immediately before use. This precaution is especially important in the case of the dilute cyanide solutions because of the escaping tendency of the HCN which exists in cyanide solutions as a product of hydrolysis.

The carbon monoxide was generated by dropping concentrated formic acid on hot, concentrated sulfuric acid, the gas being stored in bottles over water and washed through dilute alkali before introduction into the tissue chamber. All mixtures of carbon monoxide or of nitrogen with oxygen were allowed to equilibrate twelve hours before analysis and use. Samples were obtained with a Bailey bottle after each experiment and were analyzed with a Henderson-Haldane gas analysis apparatus.

Illumination of the tissue in the carbon monoxide experiments was accomplished by means of a carbon arc (copper coated sunshine carbons), the applied current ranging between 20 and 25 amperes. The arc lamp was set up about three feet from the tissue chamber and the beam focused on the tissue. No heat filter was needed since the beam traversed some three or four inches of solution in the thermostat and tissue chamber before reaching the tissue.

RESULTS. 1. *Cyanide.* An excellent example of cyanide inhibition of contracture is shown in figure 2. The tissue in this experiment was a short strip of uterus from an immature rabbit and the concentration of the cyanide was M/2,000. The procedure followed in this experiment is typical of that employed in the present work. The tissue was tied and mounted in the chamber, *T*, as shown in figure 1, and allowed to come to equilibrium with the Tyrode solution, i.e., until the tone (and pendular beats if present), became constant. Adrenaline was introduced at *A* (fig. 2), and the normal response to the stimulant in aerated Tyrode solution was obtained. Washing out the adrenaline restored the tissue to its original tone level. Cyanide was introduced at *E*, and after allowing a short period of time to elapse, adrenaline was added at *B*. Complete inhibition of the adrenaline contracture resulted. The tissue was washed with fresh Tyrode solution at *c*, and the normal response to adrenaline

in aerated Tyrode solution again obtained; this procedure served to demonstrate the reversibility of the cyanide action.

FIG 2.

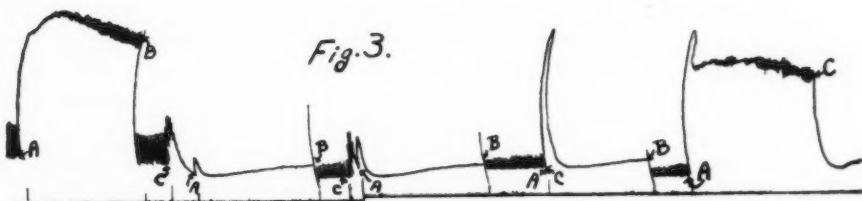
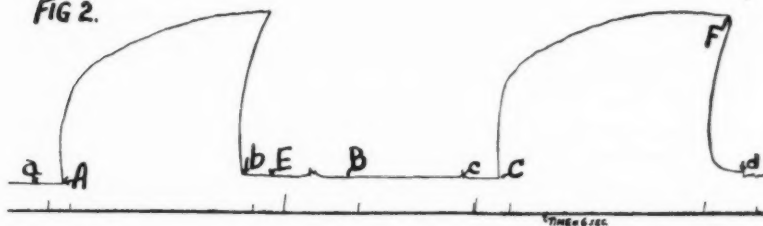


Fig. 2. Rabbit uterus. At A, B, and C, adrenaline was added to make a final concentration of 2×10^{-6} . At a, b, c, and d, tissue was washed with bicarbonate Tyrode (three changes each). At E and F, NaCN, neutralized and buffered was added to make the final concentration M/2000. Time marked in 6 second intervals on all records.

Fig. 3. Rabbit intestine. Effect of varying time between addition of cyanide and drug. At A, acetylcholine was added to make a final concentration of 2×10^{-6} . At B, tissue was washed with bicarbonate Tyrode (three changes each). At C, NaCN was added to make the final concentration M/2000.

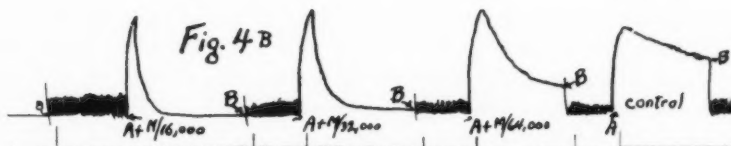


Fig. 4A and 4B. Rabbit intestine. Effect of varying the concentration of NaCN added simultaneously with the drug. Legend as in figure 3. First and last curves are controls, obtained without the addition of NaCN.

We have never succeeded in obtaining such cyanide inhibition in the case of uteri from sexually mature animals. Cyanide failed to inhibit the adrenaline contracture of such uteri regardless of the concentration, although intestinal strips from these same animals gave the usual cyanide inhibition. This effect was also observed when carbon monoxide was used instead of cyanide as the inhibitor.

One of the most striking points in experiments on cyanide inhibition is the extreme rapidity with which the cyanide penetrates the tissue and produces its effect. It was found that maximum inhibition was obtained if two to three minutes were allowed to elapse between the addition of the cyanide and the stimulating reagent. With simultaneous addition of cyanide and drug the first effect is typically a contracture of the same amplitude as that produced by the addition of drug to the unpoisoned preparation. However, within thirty seconds or so, definite inhibition of the contracture sets in and the tone falls to its original level (see figs. 3 and 4). This lag doubtless represents the time necessary for diffusion of the cyanide to the catalytic active centers.

This is particularly well demonstrated in curve *C*, figure 4. This figure shows the effect of decreasing the cyanide concentration added simultaneously with acetylcholine; even $M/64,000$ cyanide added in this manner inhibits the contracture more than 50 per cent. The high activity of the cyanide is strikingly shown in figure 5. Here the cyanide was allowed an optimal time for penetration before the addition of the acetylcholine (2.5 min.). From the record it may be seen that concentrations of cyanide as low as $M/200,000$ produce a definite inhibition as compared with the control record made with unpoisoned tissue. It should be pointed out that when such dilute cyanide solutions are used the concentrations may be only 10 to 50 per cent of the stated values because of the blowing off of HCN by the steady stream of air bubbles forced through the Tyrode solution. Discrepancies in the inhibiting effect of the various concentrations of cyanide in the series shown in figure 5 may be attributed to this uncontrollable factor. Figure 5 also demonstrates that it is possible to inhibit differentially the initial pendular-like contracture and the sustained tonic contracture of the strip by cyanide.

In seeking to determine the point of action of the cyanide its effect on the contractures produced by barium and potassium chlorides was studied. These salts presumably stimulate the contractile mechanism directly without the intervention of nerve endings, and it is interesting to see that contractures produced by these stimulants are inhibited by cyanide much the same as are those produced by acetylcholine (see figs. 6 and 7).

Since sodium cyanide is a reducing agent its inhibitory effect may possibly be attributed to this factor; if this were the case other strong reducing agents should show similar inhibitory properties. Two experiments with $NaHSO_2$ which is a strong reducing agent showed no such

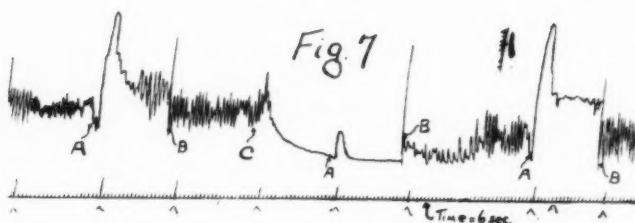
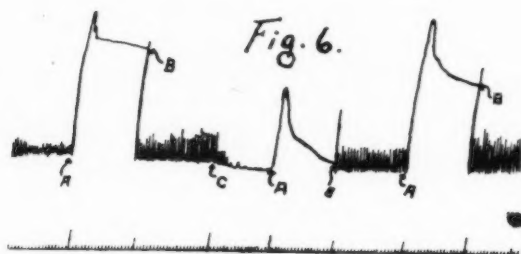
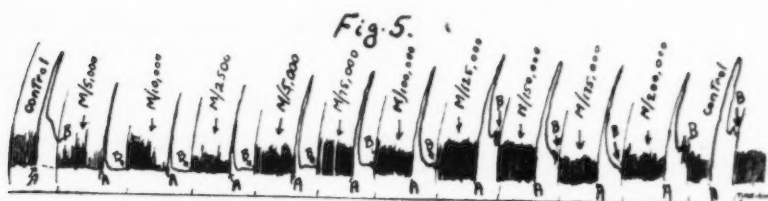


Fig. 5. Effect of varying concentration of NaCN. The drug is added at a time sufficiently long after the addition of the NaCN to ensure maximal inhibitory effect. Phosphate Tyrode solution was used. Legend as in figure 3.

Fig. 6. Cyanide inhibition of barium contracture. At A, BaCl_2 was added to make a final concentration of M/2000. At B, triple wash with phosphate Tyrode solution. At C, NaCN was added to make a concentration of M/2000.

Fig. 7. Cyanide inhibition of potassium contracture. At A, the KCl content of Tyrode solution was increased tenfold. B and C are as before.

Fig. 8. Hydrogen sulphide inhibition of acetyl choline contracture. At A, B, and C, acetyl choline was added to make a final concentration of 2×10^{-6} . At a, b, and c, triple wash with bicarbonate Tyrode solution. At E, addition of Na_2S to make the maximal concentration of H_2S approximately M/2000.

inhibitory action even in relatively high concentrations (M/500). The inhibitory action of cyanide is therefore doubtless not dependent on its reducing power alone.

2. *Hydrogen sulfide.* Sufficient sodium sulfide was added to bicarbonate Tyrode solution to yield hydrogen sulfide in a concentration of M/1,000. Due to the complex hydrolysis of Na_2S in a solution containing other salts, and to the shift in the equilibrium caused by the increase in pH of the bicarbonate Tyrode solution during the experiment, as well as to the actual loss of H_2S blown off by the aerating gas bubbles, the actual concentration to which the tissue was exposed must have been more nearly M/10,000 than M/1,000. In any event with this concentration of H_2S it was possible to inhibit the contracture in intestinal strips caused by acetylcholine, as may be seen in figure 8. Two experiments with immature uterus strips gave similar results.

3. *Carbon monoxide.* The same type of inhibitor technique was employed in experiments with carbon monoxide as in those with cyanide and hydrogen sulfide. A record was obtained of the response of the strip to a given concentration of acetylcholine or other reagent in the presence of air. After washing, the fresh Tyrode solution was then saturated for 15 to 20 minutes with a mixture of carbon monoxide and oxygen, usually in the ratio of about 9 to 1 respectively. The same concentration of acetylcholine was then added. Invariably the contracture curve was very much lower than the air control and tended to drop off rapidly. During this decline of the contracture the tissue was illuminated by means of the arc light. This resulted in an abrupt increase in the height of contracture which persisted throughout the period of illumination. As soon as illumination was discontinued the tissue again relaxed to its original low tonus level. Several such periods of illumination were usually performed before washing the tissue with fresh Tyrode solution and in each case a striking light effect was obtained. Immediately after the period in carbon monoxide, another control experiment was performed in air to demonstrate that the effects are reversible.

The fact that a light reversal was obtained is evidence that the inhibition of contracture with carbon monoxide is not a mere asphyxia produced by inadequate oxygen supply to the tissue. Nevertheless a separate control of this point was always made after the carbon monoxide record had been obtained. For this purpose the Tyrode solution was saturated with a mixture of nitrogen and oxygen, the partial pressure of the oxygen being adjusted so that it would be somewhat lower than in the case of the carbon monoxide-oxygen mixture. As can be seen from figure 9 and figure 10, the response of the tissue to acetylcholine and to barium chloride under these conditions was very similar to the response in air; there is little or no inhibition and illumination has no effect. This routine seems also to

Hence we must conclude that in all probability it is an oxidative catalysis which is required in the normal response of the tissue and which is blocked by the carbon monoxide.

While it is known from the work of Garry (1928) and others that exclusion of molecular oxygen blocks smooth muscle contracture the present work stresses the fact that it is activated oxygen that is needed. Thus in figure 11, the strip was subjected to a gas mixture containing 14.6 per cent oxygen and 85.4 per cent carbon monoxide. Nevertheless in spite of this high concentration of molecular oxygen, block resulted. This figure also shows very impressively the rapidity with which active oxygen is made available to the tissue during illumination and with which discontinuation of illumination results in rapid return to block.

4. *Urethane*. Since cyanide and carbon monoxide block the contractile mechanism in smooth muscle we wished to determine whether substrate activation might also be involved. If this were the case, narcotics would similarly block the response of smooth muscle to drugs.

Employing urethane as a typical narcotic it was found that little inhibitory action was exerted on the contractile mechanism until concentrations as high as one per cent were reached. Inhibitions with substances in such high concentrations, however, cannot be ascribed to a catalytic mechanism. Making the solution one per cent with urethane greatly increases the osmotic pressure of the Tyrode solution. Such an increase in osmotic pressure in itself (according to Dale, 1913) suffices to inhibit the action of drugs on smooth muscle. We found that simple increase in the NaCl content of the Tyrode solution produces similar results. We conclude from this that dehydrases are not directly involved in the mechanism which is so responsive to cyanide and carbon monoxide poisoning.

DISCUSSION. These experimental results require the assumption of a catalytic mechanism in some step of the contractile sequence in smooth muscle. The fundamental question then arises as to the nature of the reactions promoted by this catalytic system. One naturally tends to associate catalytic systems poisonable by traces of cyanide or hydrogen sulfide and by carbon monoxide, reversible by illumination, with the type of hemin catalysis Warburg has demonstrated to be necessary for oxygen activation in living cells. And to be sure, evidence is available that these poisons do inhibit the oxidative catalyst in smooth muscle. Schmitt and Scott (in press) found also that the oxygen consumption of stomach and intestinal tissue of the frog may be inhibited 60 per cent and that some of this decreased respiration is restored by illumination. Evans (1923) found that cyanide may inhibit the oxygen consumption of various types of smooth muscle by as much as 60 per cent. He concluded, however, that smooth muscle consumes less oxygen in tonic contraction than in a

state of relaxation. Based on the assumption that tonus is analogous to a tetanic contraction Bayliss (1928) states that a decrease of oxygen consumption is to be expected if the tension is held constant and the muscle allowed to shorten. This does not preclude the possibility, however, that the act of shortening requires oxidative energy.

It is well known that certain hormones and drugs are reducing substances. For example, adrenaline is very quickly oxidized when placed on living tissue. An explanation of our results, however, in terms of a mechanism required to oxidize the stimulating reagent is not tenable because of the fact that these poisons also block the stimulating effect of barium and potassium chlorides, which couldn't conceivably require preliminary oxidation for tissue stimulation. Furthermore, without the addition of hormones or reagents as stimulant we find that the normal tonus level is decreased by equilibrating the tissue with carbon monoxide oxygen mixtures in the dark. Illumination of the preparation then causes an immediate restoration of the original tonus level. This reversible process may be demonstrated repeatedly in one and the same strip if conditions are favorable.

It might be supposed that the cyanide and carbon monoxide block the action of a promotor of some type of reaction other than oxidations. Glutathione, for example, is claimed to be the activator for numerous enzyme reactions (Waldschmidt-Leitz, Schaffner and Kocholaty, 1931; and Borchardt and Pringsheim, 1933) and since it has been demonstrated that glutathione requires a heavy metal complement for its catalysis of oxidations, so it might be possible that such a heavy metal complement is required for its enzyme activations also.

Against this possibility that the cyanide and carbon monoxide might have the effect of poisoning a catalysis other than oxidative may be cited the experiments with pure carbon monoxide. Here the blocking effect is not decreased by illumination, whereas light reversal of carbon monoxide poisoning is prompt when the gas contains some oxygen. This can only mean that illumination changes the affinity of the catalyst for carbon monoxide and oxygen, respectively, in such a way that oxygen can again be activated and oxidative reactions may thus be promoted. Therefore, pending a closer analysis of the mechanism of the catalytic systems involved we conclude that sustained tonic contracture of smooth muscle requires a hemin-like catalytic oxidative mechanism.⁴

Garry (1928) found that the various types of smooth muscle contractions are differentially affected by oxygen lack. Similarly we find that

⁴ Fenn and Cobb (1932) have shown that carbon monoxide may be burnt by heart and skeletal muscle in the presence of sufficient oxygen. It is possible that such a mechanism may play a rôle in the present experiments but if so, the burning of carbon monoxide must take place chiefly during the period of illumination.

cyanide in low concentrations and carbon monoxide mixtures containing abundant oxygen inhibited the sustained tonic contractures without greatly affecting either the normal pendular beating of the strips or the initial high contracture of short duration which results when the strip is stimulated by drugs. With relatively high concentrations of cyanide and carbon monoxide the pendular beating may be completely inhibited, although the initial contracture of the strip when stimulated by drugs is often unaffected even in pure carbon monoxide. While it is difficult to make this inhibitor sensitivity the basis for differentiating between separate phases in the response of smooth muscle, some such differentiation is clearly indicated.

From the viscous elastic properties of smooth muscle Winton (1930) distinguishes between an initial phase of contracture associated with high tension and low viscosity (pendular beats), and a second phase associated with low tension and high viscosity (tonus changes). It is possible that these phases may eventually be interpreted in terms of oxidative processes, and that the inhibitor technique may play an important rôle in the solution of the problem. The data at present, however, are too meager to permit such an analysis.

SUMMARY

1. A modification of the Trendelenburg apparatus for recording movements of excised smooth muscle strips, which permits the tissue to be brought into equilibrium with gas mixtures of known composition, is described.

2. In the uterus and intestine strips of the rabbit, cyanide, hydrogen sulfide, and carbon monoxide inhibit tonic contractures normally caused by drugs and chemical stimulants. The inhibition caused by carbon monoxide is reversed during illumination provided sufficient oxygen is present. Urethane exhibits no inhibitory effect on such contractures.

3. Differential inhibition of smooth muscle contractile mechanisms is discussed and it is concluded that a catalytic mechanism, probably of a hemin nature, is required in some step of the contractile sequence in smooth muscle.

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COMPARATIVE STUDIES OF GONAD-STIMULATING HORMONES

III. EFFECTS OF PROLONGED INJECTIONS IN IMMATURE RATS

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In a previous communication (Fluhmann, 1933b) the effects induced in five-day experiments in the sex organs of immature female rats by an extract of human pregnancy blood, were compared to those brought about by a preparation made by a similar method from sheep anterior hypophyseal tissue. It was then shown that the pituitary extract was much more effective than the pregnancy blood in inducing increases in ovarian weight, but less capable of affecting uterine growth. The histologic examination of the ovaries also showed significant differences, the anterior hypophyseal preparation resulting in the formation of large numbers of small corpora lutea and many follicles undergoing chromatolytic or connective tissue degeneration, while the ovaries of the rats receiving pregnancy blood extract showed a few large corpora lutea, lutein cysts and developing graafian follicles with a notable absence of the atretic structures seen in the other case. The present study deals with a comparison of the changes induced in immature female rats by these two extracts when the period of administration was prolonged from 5 to 10, 15, or 20 days.

TECHNICAL PROCEDURES. The methods of preparing the two extracts were essentially the same, and as previously described (Fluhmann 1932a, 1933b) consisted of treating the original material with alcohol, extracting the resultant precipitate with a sodium acetate-acetic acid buffer, pH 4.2 to 4.6, and obtaining the active substance by reprecipitation with ethyl alcohol. Each injection consisted of 0.25 cc. of the required dilution of each preparation and was given subcutaneously twice daily except on intervening Sundays, when a single injection of 0.5 cc. was administered. In each series from 16 to 30 rats were used, and groups of from 4 to 11 sacrificed every 5 days. The total period of administration of the extracts was limited to 20 days (except in one instance) in order to complete the experiments as much as possible before the age when sexual maturity normally occurs. Since the rats were 20 to 23 days old when the injections

were begun the oldest were thus 43 days at the termination of each study. In 39 untreated controls it was found that 75 per cent were over 45 days old when the vaginal introitus was established, and the youngest were 36 days of age.

The organs were weighed on a torsion balance correct to 1 mgm. The ovaries were carefully dissected free from their bursae and the fallopian tubes, under a dissecting microscope. The uteri and fallopian tubes were weighed together, but were first slit longitudinally and firmly pressed between blotters to eliminate any accumulated fluid.

The control figures were obtained from a series of 67 immature rats, which were divided into two age groups and again subdivided into smaller categories according to the body weight (table 1). The average gross weights of the organs were reduced to terms of "100 units of body surface"

TABLE 1
Organ and body weights of 67 immature control rats

NUMBER OF RATS	AGE	BODY WEIGHT	AVERAGE GROSS WEIGHTS		$\frac{\text{WEIGHT} \times 100}{\text{BODY SURFACE}}$	
			Uterus	Ovaries	Uterus	Ovaries
	<i>days</i>	<i>grams</i>				
9	25 to 27	23-28	0.031	0.011	0.031	0.011
10	25 to 27	29-32	0.030	0.012	0.027	0.011
9	25 to 27	33-37	0.034	0.012	0.028	0.010
6	25 to 27	38-42	0.037	0.013	0.028	0.010
6	30 to 45	40-50	0.028	0.012	0.020	0.008
10	30 to 45	50-60	0.032	0.013	0.020	0.008
9	30 to 45	60-70	0.042	0.014	0.024	0.008
8	30 to 45	70-80	0.079	0.014	0.039	0.007

and the per cent increase or decrease in organ weight was calculated from these figures. The body surface area was computed from the formula $11.34 \times (\text{body weight})^{2/3}$.

Pregnancy blood extract. Although a number of experimenters have previously reported on the effect of prolonged injections of ovary-stimulating preparations made from human pregnancy urine (Zondek, 1931; Katzman, 1932, and others), apparently no systematic study of the resulting ovarian weights was conducted prior to those of Fluhmann (1932b) with a pregnancy blood extract, and MacPhail (quoted by Collip, 1932) with a human placental extract. The present report is based on the findings in 70 rats, in addition to those mentioned in the preliminary report of this work (Fluhmann, 1932b).

The effects induced in 5 to 20 days on the weights of uteri and ovaries of immature rats are shown in table 2 which give the results of 3 series of

experiments. Although the daily dose varied in each series the same type of response was observed in each instance, but was much more marked when higher dosage was employed. A gradual increase in the weights of both uterus and ovaries as the period of administration was prolonged was very clearly demonstrated.

In all instances the injected animals failed to gain weight quite as rapidly as the controls, the body weight at the end of the experiments averaging from 60 to 63 grams as compared to the control weight of 73 grams. In several groups daily vaginal smears were made, but the results showed such variations as to lead to no definite conclusion. Histologic sections of the

TABLE 2
Effect of prolonged administration of a pregnancy blood extract on the uteri and ovaries of immature rats

SERIES	EX-TRACT NUMBER	DAILY DOSE	NUMBER DAYS INJECTED	NUMBER OF RATS	AVERAGE GROSS WEIGHTS			WEIGHT \times 100 BODY SURFACE		INCREASE	
					Body	Uterus	Ovaries	Uterus	Ovaries	Uterus	Ovaries
		cc.								per cent	per cent
A	xi	0.06	5	5	32	0.098	0.020	0.086	0.017	219	70
			10	5	38	0.142	0.023	0.110	0.018	292	80
			15	6	39	0.171	0.023	0.131	0.018	368	80
			20	6	62	0.250	0.028	0.146	0.016	508	100
B	xi	0.125	5	5	27	0.077	0.020	0.075	0.019	142	63
			10	5	41	0.186	0.029	0.138	0.021	590	162
			15	5	52	0.236	0.040	0.143	0.025	615	213
			20	5	60	0.321	0.048	0.177	0.027	633	237
C	xiii	0.25	5	6	32	0.111	0.030	0.097	0.026	259	160
			10	6	43	0.176	0.054	0.127	0.039	535	387
			15	8	59	0.280	0.089	0.162	0.052	710	550
			20	8	60	0.301	0.121	0.172	0.069	760	762

vaginae in several cases, however, showed the occurrence of mucification and this is probably the most characteristic picture occurring after prolonged administration of the pregnancy blood extract. In corroboration of the work of Loeb (1932) with human pregnancy urine in guinea pigs, no stimulation of the thyroid gland could be demonstrated either by histologic study of the organ or the determination of the rate of oxygen consumption. A 30 per cent increase in adrenal weight was observed after 20 days' injection in the animals of series C.

The histologic study of the ovaries was of particular interest, in view of the marked differences from the changes induced by sheep anterior pituitary extracts noted in the five-day experiments (Fluhmann, 1933b).

Although individual variations occurred, the most characteristic finding was of large corpora lutea, some of which contained imprisoned ova, lutein cysts representing follicles undergoing a progressive luteinization, and medium-sized developing graafian follicles (fig. 1). As in the five-day experiments, there were but few larger follicles undergoing chromatolytic degeneration. A new feature was noted, however, in the presence of large cysts lined either by a few rows of lutein cells or merely by fibrous tissue. These cysts apparently are identical to those originally described by Smith and Engle (1927) in adult rats given daily anterior pituitary implants for long periods of time.

Sheep anterior pituitary extract. Although in their original study Evans and Long (1921) described the occurrence of profound changes in the ovaries of rats injected with an anterior lobe extract for several weeks,



Fig. 1

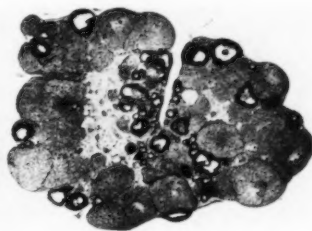


Fig. 2

Fig. 1. Photomicrograph of ovary of an immature rat injected daily for 16 days with a pregnancy blood extract. (Same magnification as fig. 2.)

Fig. 2. Photomicrograph of ovary of an immature rat injected daily for 15 days with a sheep anterior pituitary extract. (Same magnification as fig. 1.)

it would seem that no attention has been given to the effects of such extracts administered to *immature* rats for longer periods than 5 or 6 days. The present report is based on the results obtained from 4 series of rats (95 animals), each of which received an extract of sheep anterior pituitary gland of different potency.

As shown in table 3, comparable results were obtained in series D, E, and F as regards the weights of the uteri and ovaries. In all three instances there was a definite progressive increase in uterine weight as the period of injection was prolonged, although it fell short of the increase produced by the various doses of the pregnancy blood extract. As reported previously (Fluhmann, 1933a), a very remarkable phenomenon was noted in the case of the ovaries, for instead of showing a progressive increase in weight there was practically no demonstrable change from the 5-day observation at 10, 15, and 20 days, in spite of the continued administration of the same daily

doses of the extracts. The sole exception would seem to be in series E, where a 475 per cent increase in ovarian weight was noted in 18 days as compared to the 290 per cent increase in 5 days. This, however, does not represent the true state of affairs, for the high figure at 18 days was due to 2 of the 9 animals which developed heavy ovaries (0.128 and 0.140 gm.). The average ovarian weight of the other 7 animals was 0.046 gram (range of from 0.019 to 0.067 gm.), representing an average increase of only 312 per cent.

TABLE 3
Effect of prolonged daily administration of a sheep anterior pituitary extract on the uteri and ovaries of immature rats

SERIES	EX-TRACT NUM- BER	DAILY DOSE	NUM- BER DAYS IN- JECTED	NUM- BER OF RATS	AVERAGE GROSS WEIGHTS			WEIGHT \times 100 BODY SURFACE		INCREASE	
					Body	Uterus	Ovaries	Uterus	Ovaries	Uterus	Ovaries
		cc.								per cent	per cent
D	11-04	0.20	5	4	29	0.023	0.012	0.021	0.011	-25	10
			10	4	29	0.023	0.011	0.021	0.010	-25	0
			15	3	40	0.030	0.011	0.022	0.008	10	0
			22	5	54	0.068	0.015	0.042	0.009	110	10
E	02-01	0.50	5	11	34	0.089	0.047	0.074	0.039	164	290
			10	5	37	0.125	0.038	0.099	0.030	254	200
			14	5	34	0.154	0.043	0.129	0.036	361	260
			18	9	42	0.178	0.065	0.129	0.046	545	475
F	11-23	0.50	5	5	33	0.060	0.060	0.052	0.052	70	420
			10	5	47	0.089	0.054	0.060	0.038	200	375
			15	6	60	0.104	0.078	0.059	0.045	146	462
			20	5	63	0.114	0.073	0.063	0.041	162	412
G	01-31	0.50	5	11	33	0.071	0.083	0.061	0.071	118	610
			10	5	29	0.111	0.079	0.103	0.073	281	630
			15	5	54	0.194	0.173	0.119	0.106	495	1225
			20	7	74	0.278	0.285	0.139	0.142	256	1928

In series F the body weight of the animals corresponded fairly closely to that obtained when the pregnancy blood extract was given, but in the other two groups there was a marked interference with growth as shown by an average body weight of only 42 grams (control weight 70 gm.) in the animals of series E injected for 18 days, and 54 grams for those of series D treated for 22 days (control weight 77 gm.).

The rats of series G, which were given the strongest extract, showed several differences from those of the other three groups. In the first place, there was a marked interference with body growth during the first half of

the experiment, the average body weight at 10 days being 4 grams less than that of the 5-day rats. This was then succeeded by a period of excessive growth, so that a gain of 25 grams in the average body weight was recorded from the 10th to the 15th day of the experiment, and another increase of 20 grams from the 15th to the 20th day. A similar effect was observed with the ovarian weights. This extract produced a 610 per cent increase in ovarian weight in 5 days, no significant rise occurred in the next 5-day period, but on the 15th day the increase over controls was 1225 per cent and on the 20th day it was 1928 per cent. The gross weight of one pair of ovaries in this group was actually 500 mgm., and another 455 mgm.

It is unfortunate that the thyroid glands of these animals were not examined, but in subsequent experiments it has not been possible by the administration of the sheep pituitary extract to reproduce a picture resembling hyperplasia such as has been reported for the guinea pig (Loeb and Bassett, 1929). The determination of the oxygen consumption rate for immature rats given this preparation has shown increases of from 25 to 50 per cent according to dosage, however, so that it seems definite that it contains some factor which stimulates the basal metabolism.

The rats of series E sacrificed after treatment for 18 days showed a 60 per cent increase in the weight of the adrenals, while those of series G gave an 80 per cent increase after 20 days. The rats of series F gave a 40 per cent increase in 5 days, 30 per cent in 10 days, 50 per cent in 15 days, and 60 per cent in 20 days.

In each series daily vaginal smears were made on a small number of rats, but as in the animals given the pregnancy-blood extract, considerable variation occurred. In some instances, the cornified smear of estrus appeared very early and for the balance of the experiment the diestrous smear of leucocytes and nucleated epithelial cells was found. In other cases some semblance of a cycle was noted, while in still others the animals remained in estrus and gave smears of cornified cells for periods of as long as 10 days or more.

The histologic study of the ovaries from the animals given the sheep anterior lobe extract showed that the characteristic picture of large numbers of corpora lutea, many with imprisoned ova, which was observed in the 5-day experiments, was still in evidence. However, the large numbers of atretic follicles previously noted was not a prominent feature, while a few lutein cysts, occasional graafian follicles and corpora lutea in various stages of retrogression were found in specimens obtained at 10, 15 and 20 days (fig. 2). In rats given very small doses such as in series D, a number of ovaries contained many structures with degenerated ova and granulosa and a luteinization of the theca, resulting in a marked increase of interstitial tissue. Large cysts such as found in the pregnancy-blood series and following anterior pituitary implants in adult rats (Smith and Engle, 1927)

were not seen. Except for a difference in the numbers of corpora present the ovaries of the animals of series G, which increased so much in weight, gave essentially the same histologic picture as the rats of the other series. In several instances serial sections of the fallopian tubes were made but in no case were ova seen.

DISCUSSION. It is thus seen that the prolonged administration to immature rats of acid extracts of human pregnancy blood and of sheep anterior pituitary lobe accentuates the differences in the action of these two preparations. The most striking change is seen in the effect on the weight and on the histologic appearance of the ovaries.

In the case of the pregnancy-blood extract, there is a definite *cumulative* effect in producing increases in ovarian weight, the most pronounced change occurring as the period of administration is prolonged. This feature was still more clearly brought out in a previous study (Fluhmann, 1933c) when it was shown that with a fixed total dose greater increases in ovarian weight could be obtained by prolonging the period of administration.

The sheep anterior pituitary preparation at doses which produce increases in ovarian weight of less than 500 per cent in 5 days, has been shown to act very differently. No cumulative effect was demonstrated, and after reaching a maximum within a few days the ovarian weight was maintained at essentially the same level in spite of the continued injection of the same daily dose for as long as 20 days. The histologic changes in these ovaries are also of importance since the presence of luteinizing cysts, developing follicles, and regressing corpora gave evidence of continued activity even with the lack of increase in weight. These findings are of special interest as they present a distinct simile to the normal physiological process which occurs in adult female rats. Although normally the anterior pituitary gland is regularly (or cyclically) secreting a gonad-stimulating hormone, the ovaries give evidence of activity at different stages of the estrual cycle and yet maintain their gross weight with only minor variations. It may also be significant that a progressive increase in ovarian weight was only obtained with sheep anterior pituitary extract when doses were employed, as in series G, which stimulated increases in ovarian weight beyond the level normally found in adult rats. In series D, E, and F, the weights produced were essentially within the physiological limits for adults.

Although the mechanism by which these various changes are brought about is as yet unknown, a clue is to be found in the work of Loeb and Friedman (1931) who pointed out a relation between the effects induced by anterior lobe extracts on the ovaries and on the thyroid glands of guinea pigs. In preliminary studies on this question some evidence has been obtained in this laboratory which suggests that the increase in basal

metabolism stimulated by the sheep anterior pituitary extract may be the chief factor concerned in preventing a continued increase in ovarian weight in chronic experiments.

It is indeed difficult to evaluate the differences obtained from the use of the two extracts, and determine if they really signify that we are dealing with two separate sex hormones. The problem is particularly complicated by the varied effects produced by anterior lobe preparations in experimental animals and the trend of experimenters in assigning a different factor to the pituitary for each of these changes. It would seem that the final answer as to whether the gonad-stimulating hormone of human pregnancy is a pituitary product or not will only come when, and if, a chemically pure gonad-stimulating hormone from anterior lobe material is obtained for comparison. For the present, however, it seems clear that there is a marked difference in the biologic effects induced by the two products which strongly suggests that they are not identical.

SUMMARY

1. The changes resulting from the daily administration to immature rats of acid extracts prepared from human pregnancy blood and from sheep anterior pituitary glands for periods of from 5 to 20 days showed marked differences.

2. The pregnancy-blood extract had a *cumulative* effect on the ovaries, which progressively increased in weight as the period of injection was prolonged.

3. The daily injection of sheep anterior pituitary extract in such doses that the ovaries increased from 100 to 500 per cent in weight in the first 5 days, failed to produce any further marked increase in ovarian weight when the period of administration was prolonged to from 10 to 20 days. However, the ovaries showed very great increases in weight when much larger daily doses were employed for 15 or 20 days.

4. Significant differences were noted in the histologic picture of the ovaries of the rats given pregnancy-blood extract from those receiving the sheep anterior hypophyseal preparation.

5. The sheep anterior lobe extract produced increases of from 60 to 80 per cent in the weight of the adrenals in 20 days, whereas the pregnancy-blood extract only induced a 30 per cent increase.

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A STUDY OF THE SECRETORY PRESSURE OF THE LIVER

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We have conducted experiments to determine the relative stimulating effect of secretin and purified bile salts upon the secretory pressure and secretory rate of the liver after the onset of the resorption phase. We have investigated also, in acute experiments, the effect of partial and complete obstruction of the common bile duct upon the rate of flow of bile. Observations of a somewhat similar nature have been reported by Herring and Simpson (1907), Simpson (1910-11), Mitchell and Stifel (1916), Mann and Foster (1918-19), Still, McBean, and Ries (1931), and Regan and Horrall (1932).

EXPERIMENTAL METHOD. The dogs were fasted for 24 to 48 hours. To induce anesthesia sodium barbital was injected intramuscularly (320 mgm. per kgm.). In some of the earlier experiments the barbital was administered intravenously under temporary ether anesthesia. A high abdominal incision was made along the linea alba about two and one-half inches in length. The cystic duct was clamped with a hemostat, the common duct was cannulated near its intestinal end with a glass cannula and the abdominal incision closed. Each animal was kept at 37° to 39°C. with artificial heat throughout the experiment.

To record secretory pressure, rate of bile flow, or both, the common duct was connected to a manometer and an electric drop recorder by a T-tube. Either could be removed from the system by manipulation of appropriate stopcocks. The drop recorder could be raised or lowered so as to measure the effect of pressure upon rate. We constructed our manometer so that adjustment to zero pressure was possible with the avoidance of a capillary error. The time of starting the observation at zero pressure was recorded, and readings were taken on the graph paper using time as the abscissa and pressure as the ordinate. Intervals between readings varied between 0.25 and 15 minutes. In experiments where volume flow was considered drops were recorded until a constant flow had been maintained for two hours before experimental conditions were applied. This was necessary because it was found that after the operation there was a steady increase in the rate

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of flow at zero pressure for some time, probably due to recovery from the effects of the preparation of the animal. It may have been lack of observance of this phenomenon that caused Simpson to state that an increased pressure of 170 mm. bile led to an increased flow of bile.

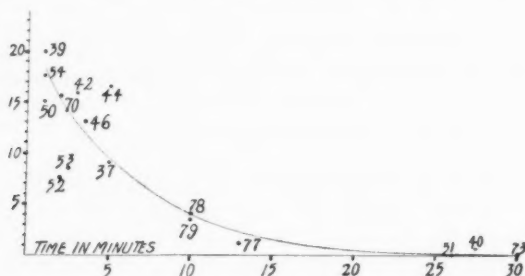


Fig. 1. The effect on the secretory pressure of the liver (dog) of the intravenous injection of sodium dehydrocholate (50 mgm. per kgm.) at various intervals after the onset of the resorption phase. Ordinate—per cent increase in the secretory pressure. Numerals refer to experiment numbers.

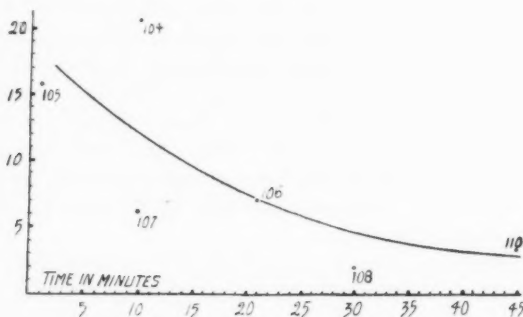


Fig. 2. The effect on the secretory pressure of the liver (dog) of the intravenous injection of secretin (1 mgm. per kgm.) at various intervals after the onset of the resorption phase. Ordinate—per cent increase in the secretory pressure. Numerals refer to experiment numbers.

RESULTS. When the bile pressure was allowed to develop by its own secretion two general types of curves were noted. In one a plateau is gradually reached, the maximum height is maintained for 15 to 30 minutes and then the pressure gradually recedes. In the other the maximum pressure is held for a shorter time and after a period of from 6 to 10 hours during which some resorption takes place the pressure again rises but never again reaches its first maximum. There may be gradations between the two.

No explanation is offered for the difference in the two types, but the absence of a single uniform type precludes certain approaches to problems.

The average maximum biliary pressure in forty experiments was 285 mm. bile. The highest pressure observed was 329 mm. and the lowest was 224 mm. This average is about 15 mm. lower than the average reported by Herring and Simpson, and agrees very well with the data reported by Mitchell and Stifel, and Still, McBean and Ries. However, if the capillary error of the apparatus used by Herring and Simpson be taken into account, their average pressure would be about 275 mm.

Our method of dealing with bile flow shows that there is no significant change in the rate of flow with increased pressure up to about 200 mm. Additional data show that there is little change in the rate of flow until the pressure is raised to within about 50 mm. of its maximum, when a sharp falling off occurs.

If sodium dehydrocholate is injected intravenously when the maximum pressure is reached, there is an additional rise of bile pressure. If injected 30 minutes after resorption starts there is no material change in the curve. Between these two extremes there is a gradual variation in the response to bile salts (fig. 1).

The secretin used in our experiments was from sample no. 372 (Still, 1930). We used a dose of 1 mgm. per kgm. In general the effect resembled that of sodium dehydrocholate, except that the stimulating action was effective over a longer period of the resorption phase. Figure 2 shows the effect of secretin during the resorption phase on the secretory pressure curve of the liver.

CONCLUSIONS

1. The average maximum secretory pressure of the liver of forty dogs that had been fasted 24 to 48 hours was 285 mm. bile.
2. Increase in secretory pressure of the liver had no significant effect upon the rate of bile flow until within a few centimeters of the maximum pressure, at which time a decrease in rate of flow occurs.
3. If bile salts are injected intravenously within about 20 minutes from the beginning of the resorption phase in dogs (which have developed their maximum biliary secretory pressures) there will be effected a further rise in the secretory pressure. At a later time, however, there is no observable effect. The intravenous injection of secretin yields similar results, but the effective time after the onset of resorption is longer than in the experiments with bile salts.

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